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THE MONKEY (*MACACA MULATTA*) AFTER HEMISECTION AND SUBSEQUENT TRANSECTION OF SPINAL CORD*

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(Received for publication January 23, 1943)

INTRODUCTION

THE REFLEX picture resulting from hemisection followed at a suitable interval by transection has been described by Fulton and McCouch (1) in the case of a single baboon and the cord potentials in the monkey have been discussed by the present authors (5). Since certain of the results we reported in 1940 have not been confirmed uniformly in a larger series, we are impelled to reopen the subject.

RESULTS

In accord with earlier results (1, 5), all of our fifteen animals recovered reflex activity more rapidly upon the side of previous hemisection. On the other hand, internuncial potentials recorded from dorsal or dorsolateral surface leads yielded responses that rarely showed significant differences between the two sides. When asymmetry did occur, it was far less marked in the potentials of interneurons than in the corresponding reflex actions. These points are indicated by the difference in threshold between cord potential and ipsilateral flexor reflex presented in Table 1 in the form of a ratio. In the previously monoplegic extremity this may approximate unity. On the previously intact side, the value for the threshold of the internuncial potential divided by that for the ipsilateral flexor reflex lies between 0.51 and 0.

Table 1. Ratio of Thresholds $\frac{\textit{Cord Potential}}{\textit{Reflex}}$

Date	Chronic	Acute	
3/ 7/39	1.0–0.89	0.42	Both legs dissected
6/20/40	0.47	0	" " "
12/26/40	0.95	0.51–0.36	Legs not dissected
3/ 4/41	0.74	0.37	" " "

A high threshold for the crossed cord potential may occur on either side or upon neither and is frequently associated with a lesser rise in threshold on the ipsilateral side. In one instance, replacement of stimulating electrodes

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at a point farther from the cut end of the root lowered both ipsilateral and crossed thresholds to a common level comparable to that obtained from the root of the opposite side.

In terms of crossed inhibition the evidence of asymmetry is scarcely less

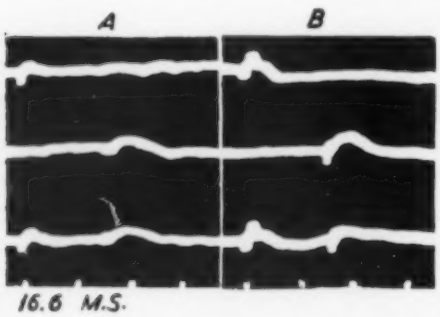

FIG. 1. A: Lead from the right dorsal surface of the cord. Conditioning shock of 110 arbitrary units of stimulus strength to a left dorsal root. Test shock of 30 units to a right dorsal root; both shocks at interval of 27 msec. Note the absence of inhibition even when a weak test volley is employed. On this side there was no visible inhibition of digital flexion.

B: Lead left. Conditioning shock right 110 units: test shock left 60 units; both shocks at interval of 28 msec. Note the marked inhibition of the cord potential. The movement of digital flexion was inhibited completely with this comtion of stimuli. *Macaca mulatta* 3 kg.: right hemisection Dec. 4, 1940; transection and recording Dec. 26, 1940.

meager. This was tested on both sides in nine experiments, in only one of which was there a significant difference, the previously paretic side proving the more potent source of inhibition of both internuncial potential and reflex (Fig. 1). The more usual symmetrical situation (in this instance with shock to the crossed potential and no interaction) is illustrated in Fig. 2.

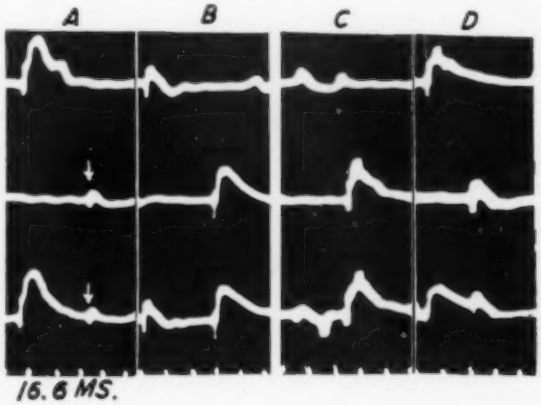

FIG. 2. A: Right dorsal lead; conditioning shock of 58 units to a right dorsal root; test shock of 58 units to a left dorsal root; both shocks at interval of 37 msec.

B: The same combination of stimuli led from the left. Shock inter- 46 msec.

C: Right lead; conditioning shock of 34 units to a left dorsal root; test shock of 18 units to a right dorsal root; both shocks at interval of 32 msec.

D: The same combination of stimuli led from the left; shock interval 29 msec. Note the evidence of spinal shock in the crossed potentials and the absence of inhibition in all four columns. *Macaca mulatta* 3 kg.: right hemisected May 22, 1941; transected and recorded June 10, 1941.

Far more predictable is the order of recovery of crossed reflexes. The severity of spinal shock in this field proved a serious limitation, since it was essential to record soon after transection in order to obtain the highest available degree of asymmetry. Consequently, only two of our monkeys so recorded developed a crossed response. In both instances it was elicited in the musculature of the previously paretic limb by a stimulus on the previously intact side. Of special interest is an animal recorded 41 days after hemisection but only a few hours after transection. This is the only instance we

have observed of the presence of a crossed reflex giving gross movement in the absence of any visible ipsilateral response. There had been no dissection of the legs. Save for a laminectomy and section of a dorsal root on each side, the animal was intact below the level of transection. Various strengths of stimulus were employed of which a wide range yielded flexion of the contralateral toes. Yet at no time was there movement or visible muscular con-

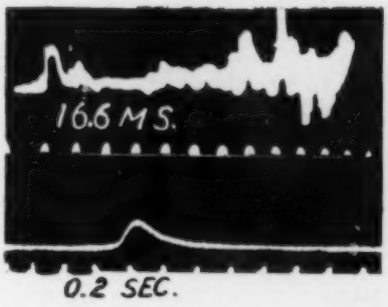

FIG. 3. Crossed extension of leg of chronic side. Upper record: Cord potential from left dorsal lead. Lower record: myogram of right quadriceps femoris. The records are not temporally aligned. The stimulus induced a single volley in the left sciatic nerve including delta fibers. Presumably the abrupt onset of the potential is associated with the ipsilateral flexor reflex; the later, progressively increasing response with the crossed extension recorded in the myogram. Time in cord potential in units of 16.6 msec. Time in myogram in units of 0.2 sec. Tension developed in reflex 130 g. *Macacus mordax* 3.3 kg.: right hemisection Oct. 18, 1939; transection Nov. 9, 1939; recording Nov. 14, 1939.

traction upon the side of stimulation. Only by electrical records from muscle was a faint ipsilateral reflex demonstrable. In sharp contrast was the response from the root of the previously paretic side: sharp contraction of ipsilateral semitendinosus and no visible crossed response.

The other case of crossed contraction occurred five days after transection, when extensor motoneurons of the previously hemiplegic leg had recovered sufficiently to give the crossed response of quadriceps femoris shown in Fig. 3.

In these two animals the relation of the type of the crossed reflex to the interval after transection raised the question whether in monkey as in cat (4) crossed flexion may recover from spinal shock earlier than crossed extension. This suspicion was confirmed in a third monkey in which the crossed reflexes were elicited by stimulating electrodes in the pads of the feet almost daily throughout the period of asymmetry. Crossed flexion of toes occurred 5 hours after transection and persisted for 7 days. It responded to single induction shocks as well as to repetitive stimulation. Crossed extension of proximal joints with flexion of digits was first obtained two days later. During the following week the crossed extensor response increased and crossed flexion of digits decreased progressively. Nine days after transection crossed extension occurred without movement of digits, although digital flexion was still readily induced as an ipsilateral reflex. As in the two previous cases, the crossed reflexes were more readily elicited in the chronic extremity from a stimulus on the acute side than vice versa.

DISCUSSION

The monkey in which hemisection is followed after a suitable interval (1) by transection offers a useful preparation for the localization of spinal shock. So far as ipsilateral reflexes are concerned, the result was predictable from

Sherrington's fundamental experiment of successive transections, of which the second failed to induce a degree of depression comparable to that ensuing after the first (6). In this regard we merely confirm for the monkey the result of Fulton and McCouch (1) in a single baboon. Reflexes are less depressed by transection on the side of the previous hemisection because the majority of the descending axons which facilitate their arcs had been severed by the preceding hemisection and thus a longer interval for recovery was obtained.

The crossed effects are less obvious. Here the result hinges upon the relative depression of the various neurons in a complex reflex path. In previous papers (2, 7) evidence has been advanced for the proposition that in the monkey spinal shock depresses the cells of the ventral horn (presumably the motoneurons) far more deeply than the interneurons situated more dorsally. This conclusion is supported by the present findings. If the last cells to recover be the motoneurons, crossed reflexes should find their earliest motor expression upon the side of previous hemisection where recovery is more advanced and in those units which show least depression. Such results as we have confirm this expectation. Thus digital flexion, which is the first ipsilateral reflex to return is likewise the first crossed response.

In the case of crossed excitatory reflexes just considered, it is essential for the interneurons on the side suffering the greater depression to recover sufficiently to discharge the contralateral motoneurons. With crossed inhibitory reflexes such internuncial shock may be a significant factor in the order of recovery. Another may lie in the high susceptibility to inhibition of the motoneuron when its excitability is low (3). Both influences favor inhibition from a source on the chronic side of previous hemisection acting upon the motor cells of the more depressed side which has suffered almost its entire quota of shock after transection. Here again our single significant instance of such asymmetry accords with expectation.

Consonant with the evidence of the relatively light degree of shock to interneurons is the frequent absence of asymmetry in their potential, even in its crossed component. A high threshold for the crossed potential, though exceptional, may occur on either side and in one instance was lowered by adjustment of stimulating electrodes. We would not imply, however, that it never occurs as a result of asymmetry in depression of interneurons. That such was actually the case in the instance cited in a previous paper (5) is strongly suggested by its long latency in that animal. In a situation in which the result is determined in part by the magnitude of the volley in crossing neurons, in part by the depression of the cells on which they impinge, either of these opposing factors may prove dominant and hence it is scarcely surprising that the threshold should prove unpredictable.

Summary

A series of 14 monkeys (*Macaca mulatta*) and one *Macacus mordax* have been studied after hemisection and subsequent transection of the spinal cord.

Reflex recovery was always more rapid in the previously paretic extremity.

Three animals developed crossed reflexes on the chronic side in response to stimulation on the acute side. Crossed flexion of digits was recorded a few hours after transection; crossed extension of the leg two or more days later.

Crossed inhibition may be more effectual when driven from the chronic side affecting the acute side than vice versa.

Only exceptionally is asymmetry reflected in internuncial potentials; never to the degree obtaining in the corresponding reflex responses.

The significance of the asymmetry observed is discussed in relation to spinal shock.

REFERENCES

1. FULTON, J. F., and MCCOUCH, G. P. The relation of the motor area of primates to the hyporeflexia ("spinal shock") of spinal transection. *J. nerv. ment. Dis.*, 1937, *86:* 125–146.
2. HUGHES, J., MCCOUCH, G. P., and STEWART, W. B. Cord potentials in spinal shock: multiple stimuli. *J. Neurophysiol.*, 1940, *3:* 146–150.
3. MCCOUCH, G. P., HUGHES, J., and STEWART, W. B. Crossed inhibition of the flexor reflex in the spinal mammal. *J. Neurophysiol.*, 1941, *4:* 547–554.
4. MCCOUCH, G. P., SNAPE, W. J., and STEWART, W. B. Note on reflex thresholds in the cat during spinal shock. *Amer. J. Physiol.*, 1935, *111:* 263–271.
5. MCCOUCH, G. P., STEWART, W. B., and HUGHES, J. Cord potentials in spinal shock: crossed effects in monkey, *Macaca mulatta*. *J. Neurophysiol.*, 1940, *3:* 151–155.
6. SHERRINGTON, C. S. *Integrative action of the nervous system*. London, Constable and Company, 1911.
7. STEWART, W. B., HUGHES, J., and MCCOUCH, G. P. Cord potentials in spinal shock: single volleys. *J. Neurophysiol.*, 1940, *3:* 139–145.

THE EFFECT OF INSULIN HYPOGLYCEMIA ON CONDITIONED REFLEXES

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KESSLER and Gellhorn (4) showed recently that convulsions induced by metrazol or by electroshock led to the restoration of previously inhibited conditioned responses. After several injections of metrazol or after electroshock conditioned responses which had been completely inhibited by lack of reenforcement were restored up to about 70 per cent. The period of restoration lasted for several days during which conditioned responses gradually declined. The effect could be shown repeatedly in the same animal.

An investigation of the effect of hypoglycemia under similar conditions seems to be of great interest for 3 reasons: (i) to increase our knowledge of the effects of insulin hypoglycemia on the brain in chronic experiments; (ii) in order to determine whether the restoration of inhibited conditioned responses results from convulsions only; (iii) in order to obtain, if possible, further material suggesting the fundamental similarity of the physiological mechanisms involved in various forms of shock therapy (1, 2, 3).

The experiments reported in this paper show indeed that insulin hypoglycemia may restore, to a surprising degree, previously inhibited conditioned responses.

METHOD

The experiments were performed on more than 40 rats, mostly male, of 200 to 250 g. weight. The same apparatus as described by Kessler and Gellhorn (3) was used. It consisted of two compartments separated by a low partition. The floor of the compartment was made of copper wire grid which could be charged through a General Electric Variac. A few trials were sufficient to cause the rat to jump from one compartment into the other in response to the electric shock (about 40 V.) applied to the grid. The response consisted of two integrated movements: first, the rat jumped across the partition, then turned around completely and again faced the partition in readiness for another jump.

After the unconditioned response (u.r.) had been established in a few trials the conditioning process was carried out as follows: a bell was sounded for two seconds and was followed by an electrical shock. The sound of the bell was continued to the end of the electrical stimulation. To avoid fatigue, not more than 25 to 30 reenforced conditioning stimuli (bell plus shock) were applied in one session. The experiments were performed daily and in general 70 to 120 stimuli (bell plus shock) were adequate to establish a conditioned response of 90 to 100 per cent. This response was then maintained for three successive days, to insure a thorough retention of the conditioned response (c.r.). This was accomplished by subjecting the animals to 7 to 12 conditioned stimuli (c.s., bell) reenforced by unconditioned stimuli (u.s., shock) before applying the test series of 10 nonreenforced c.s.

Hereafter the c.r. was inhibited by the daily application of 10 unreenforced c.s. (inhibition by lack of reenforcement in the sense of Pavlov). However, this test was always preceded by one or, in the majority of the experiments, two reenforced stimuli. In the latter case one was applied while the animal was in compartment A and the other in the compartment B in order to avoid the formation of positional habits.

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* Aided by a grant from the John and Mary R. Markle Foundation.

This procedure led with great regularity to a loss of 80 to 90 per cent of the c.r. in about 3 to 4 days. After this level had been established for at least 6 days, the rats were subjected to insulin hypoglycemia in order to attempt a restoration of the c.r.

Four to 10 units of insulin (Lilly)* were injected intraperitoneally in two divided doses at an interval of one hour. After the hypoglycemic effects described later had taken place, normal blood sugar and behavior were reestablished by injection of 5 cc. of 8 to 12 per cent glucose.

Results

Since insulin hypoglycemia tends to restore inhibited c.r. it was first necessary to determine whether or not inhibited c.r recover spontaneously. Numerous experiments performed by Kessler and Gellhorn as well as by the present authors show that this is not the case. The following series of experiments illustrates this finding.

Three rats in which the c.r. had been established and maintained at 90 per cent for 3 days were tested for a period of 25 days daily by 10 nonreenforced c.s. which were preceded by two reenforced c.s. This procedure led to an inhibition of the c.r. in 3 to 5 days. Hereafter the c.r. remained at a level of 0 to 10 per cent for 25 days. Only on one day was it found in one animal that the c.r. rose to 20 per cent. On the basis of these and similar experiments it is concluded that a recovery of more than 20 per cent is significant.

The main group of experiments comprises 22 rats in which the c.r. to the bell had been inhibited by lack of reenforcement. The administration of insulin resulted in a restoration of the inhibited c.r., to varying degrees, lasting several days to several months. In 5 animals these effects could not be obtained but in most instances these animals died before the insulin test could be repeated several times. It is worthy of note that in the last 11 animals no failure was observed.

Two factors appear to be of importance for the recovery of inhibited c.r. by insulin hypoglycemia: (i), the degree of cerebral depression seems to play a role; (ii), the results seem to depend to a certain extent on the method of inhibiting and testing the animal. It was mentioned in the preceding section that inhibiting and testing was done by having a period of 10 nonreenforced c.s. preceded by one or two reenforced stimuli (bell+shock). It is our impression that the use of two reenforced stimuli is of definite advantage. This procedure does not prevent gradual abolishment of formerly established c.r. and still maintains the animal during the period of complete inhibition of the c.r. in a sufficient degree of alertness to permit recovery. It is with this method that a 100 per cent success was achieved in the last 11 animals although in control animals not subjected to insulin the c.r. never recovered spontaneously.

The description of a few characteristic experiments will show that the recovery depends also on the severity of the hypoglycemic syndrome. Hypoglycemic symptoms occurring within two hours following the injection of insulin may be classified into three convenient groups: (i) insulin depression characterized by absence of spontaneous movements, diminished tone of

* Kindly supplied by Eli Lilly and Company.

extremities, slow righting reflexes and salivation. At this stage the rat reacts to pain but not to slight pressure; (ii) insulin coma in which righting reflexes and reactions to painful stimuli are abolished; (iii) tonic-clonic hypoglycemic convulsions.

Ordinarily, these stages followed each other in brief intervals. Occasionally, however, convulsions occurred very suddenly without having been preceded by a comatose phase.

The convulsive phase had to be terminated immediately since an attempt to keep the animal in this condition resulted in death in most in-

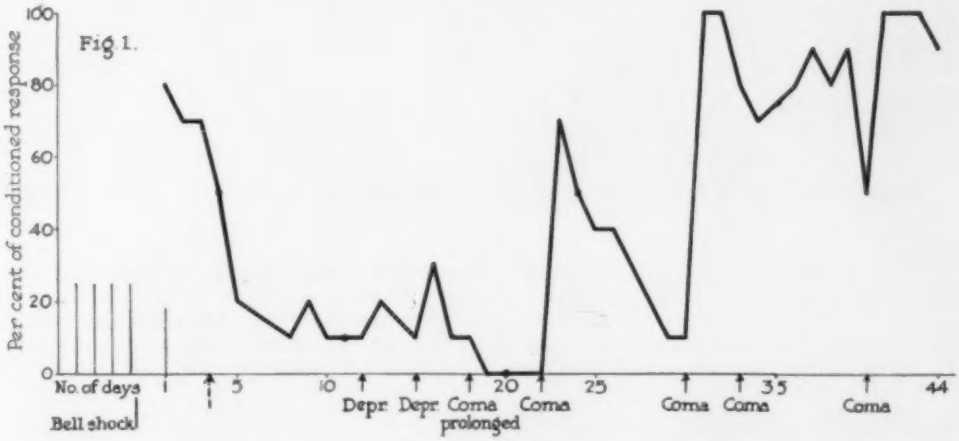

FIG. 1. Effect of insulin hypoglycemia on the restoration of previously inhibited conditioned reactions. Vertical lines at the beginning of the graph show the number of reenforced conditioned stimuli (bell plus shock) which established the conditioned response. It was maintained for three days at 70 to 80 per cent and then inhibited by lack of reenforcement. On the 12th day 4 units/kg. of insulin were given intraperitoneally, on the other days marked by an arrow 5 units/kg. were administered. The experiment showed that hypoglycemic "depressions" were unable to restore the conditioned reaction but coma caused recovery. Note the cumulative effect of three comas given between the 30th and 40th day.

stances. The coma was allowed to persist for about 7 minutes since after this period convulsions ensued frequently. The period of depression was of similar duration.

In Fig. 1 it is shown that the injection of insulin leading to a depression on the first two occasions did not alter significantly the c.r. of the inhibited rat. Several days later insulin was again injected and resulted in a deep coma which was without effect on the c.r. However, 4 days later when the injection of insulin resulted again in coma the c.r. recovered up to 70 per cent. When 8 days later the c.r. had returned to the level of 10 per cent, 3 more comas were produced by insulin, the second followed the first after 3 days and the third followed the second after 7 days. The results show very strikingly a recovery of the c.r. to 100 per cent which is only slightly diminished some days later because the subsequent insulin comas tend to restore the

inhibited responses up to 100 per cent. This experiment suggests that insulin coma is more effective in restoring the c.r. than is the hypoglycemic "depression." Moreover, it appears probable that insulin coma exerts a cumulative effect on the central nervous system since the coma becomes more effective

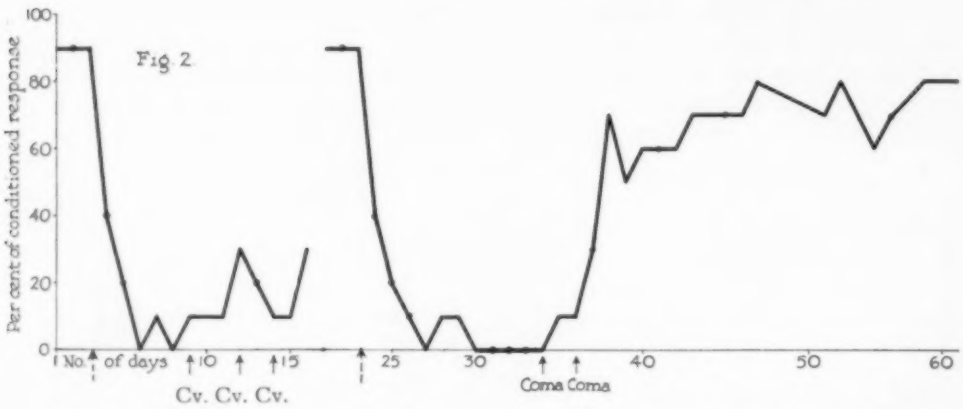

FIG. 2. Comparison of the action of insulin convulsions with that of coma. General arrangement as in Fig. 1. At the arrows 10 units of insulin per/kg. were given which resulted in convulsions on the 9th, 12th and 14th days. Insulin convulsions were ineffective. The rat was then reconditioned and hereafter inhibited. Two comas (34th and 36th day) led to a permanent restoration of the conditioned response. Cv. in figure stands for convulsion.

on repetition. By properly timing the coma it seems to be possible to permanently restore the inhibited c.r.

The first graph of Fig. 2 shows that 3 insulin convulsions did not sig-

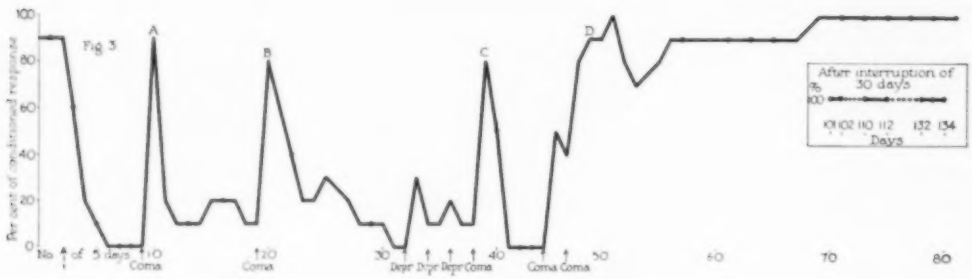

FIG. 3. General arrangement as in Fig. 1. Insulin "depressions" were ineffective whereas even a single coma caused temporary recovery. (A, B, C). Permanent recovery was affected by two comas induced on the 44th and 46th day.

nificantly alter the state of inhibition. The animal was then reconditioned and reinhibited (second graph of Fig. 2). The rat was again subjected to insulin. The first insulin injection caused slight convulsions and was not effective; the second induced a coma and was followed by a prompt recovery which lasted for 22 days until the death of the animal.

In the experiment reproduced in Fig. 3 the sequence of insulin depression and coma was reversed and under these conditions hypoglycemic depression failed likewise to restore the inhibited c.r. In this animal a single coma was sufficient to produce a marked but temporary recovery. This effect was repeated twice before the effect of hypoglycemic depression was studied. Although each insulin coma brought about a recovery of 80 or 90 per cent, even 3 insulin depressions failed to alter the c.r. significantly. A coma administered subsequently caused an effect similar to those obtained earlier in the same animal. The experiments show that insulin hypoglycemia leading to depression only was completely ineffective in this animal.

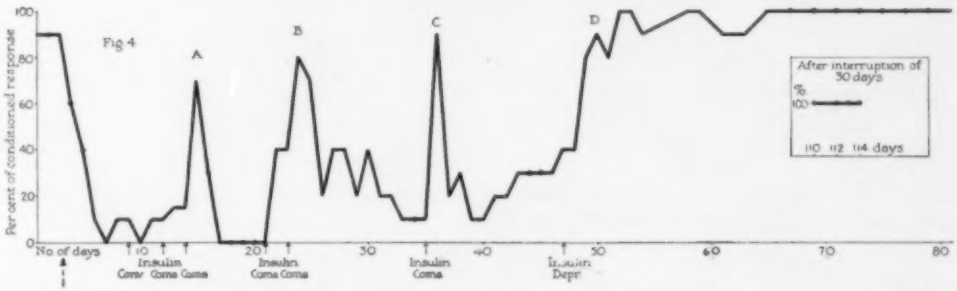

FIG. 4. Temporary and permanent effects induced by 10 units of insulin per kg. at the arrows.

After the effect of the third coma (C) had passed off insulin was injected on two alternative days leading in each case to coma. The effect was very striking. Not only did it lead to a recovery of the inhibited c.r. but this effect was also maintained for about 3 months. During this time the animal was frequently tested with 10 non-reenforced c.s which were *not* preceded by the customary reenforced stimuli. But even this procedure failed to induce any inhibition in this animal.

A similar case of permanent recovery of previously inhibited c.r. is illustrated in Fig. 4. There are, however, individual features in this experiment which are worthy of mention. The first insulin injection led to convulsions and was without effect. The second and third insulin injections produced coma which led to a temporary recovery for two days only. After this effect had completely disappeared, two more insulin comas were given which resulted in a recovery of distinctly greater duration. This cumulative effect may be responsible for the fact that a single coma given later (C) produced an effect at least as great as the first two comas had elicited. In addition, it was found that there was a gradual increase in the number of positive c.r. occurring between the 8th and 13th day after the last coma. At that time the animal was very alert and was again subjected to insulin which led only to a depression. However, this "treatment" was followed by a marked recovery which proved to be permanent. The animal was observed for more than two months following the last insulin injection. C.r. were maintained at 100 per

cent in spite of the complete lack of reenforcing stimuli. The period of testing was interrupted for one month without altering this record.

In two more animals a complete recovery was observed for 80 and 90 days respectively at which time the experiment was discontinued. In another animal repeated insulin injections led to a restitution of the inhibited reflexes for more than 30 days and then a gradual decline occurred.

The experiments prove conclusively that by properly spaced insulin comas inhibited conditioned reflexes can be permanently restored. Moreover, this effect continues in spite of the fact that the "permanently recovered" rats do not at any time receive the reenforced c.s. They jumped from one

*Table 1. Specificity of the recovery of the conditioned response after insulin coma**

Rats	Application of 5 Sound Stimuli (A) from Oscillator Followed by 10 Bell Stimuli (B)			Application of 5 Stimuli B (bell); followed by 3 Sound Stimuli A; followed by 5 Stimuli B; followed by 2 Stimuli A		
	Days	No. of Resp. to Sound A	No. of Resp. to Bell B	Days	No. of Resp. to Sound A	No. of Resp. to Bell B
11	1	0	10	1	0	10
	2	0	9	2	0	10
	3	1	10	3	1	10
13	1	0	10	1	0	10
	2	0	10	2	0	10
	3	0	10	3	0	10
14	1	1	10	1	0	10
	2	0	9	2	1	10
	3	0	10	3	0	10
15	1	0	8	1	0	10
	2	1	10	2	0	9
	3	0	10	3	0	9

* All rats had been previously conditioned only to the "bell" and not to the sound of 250 vibrations produced by an oscillator.

compartment into the other before the shock which was customarily applied after an interval of two seconds could become effective.

The observation that rats in which the c.r. had been inhibited show a restitution of this response either temporarily or permanently following insulin treatment raises the question whether this effect is due to a disinhibition of these animals or is simply the result of a generally increased excitability which induces the animal to jump in response to the bell. Two series of experiments were performed to decide this question. In the first, 3 normal rats which had not been previously conditioned were subjected to two insulin comas on alternate days and tested daily for 15 days following insulin coma. The results showed that although all these animals were tested in exactly

the same manner as the rats described in the preceding section, they did not react at all to the sound of the bell. These results, therefore, are conclusive in showing that animals which never have been conditioned before do not spontaneously respond to the sound of the bell even though they have been subjected to insulin coma. Apparently, the insulin "treatment" can become effective only after the c.r. has been previously induced and then inhibited.

In a second group of experiments the question was investigated whether the restitution of the c.r. was specific for the previously inhibited c.s. or whether a positive response would result from the application of other stimuli as well. The experiments of Table 1 show that when insulin coma had resulted in a complete recovery of the previously inhibited c.r. to the bell almost no positive response was obtained when a sound of about 250 vibrations (S-250) was substituted for the bell. Experiments on 3 more animals, not included in Table 1, showed a similar result. In two of them the animals had been inhibited to the bell and showed restitution of the c.r. after insulin. No reaction to S-250 occurred. In a third experiment in which the c.r. to bell and to S-250 had been inhibited the response was restored to both c.s. by insulin. It was found also that a light which was not previously used as c.s. was completely ineffective although in other experiments in which the rat had been conditioned to light this reaction could be inhibited and then restored by insulin. It may, therefore, be concluded that insulin coma results in disinhibition of previously inhibited conditioned reflexes.

In view of the results described in the preceding paragraphs the important question arises whether the action of insulin coma on conditioned reflexes consists only in the removal of inhibitions, or whether under the influence of insulin coma excitatory reactions which are the basis of conditioning processes are likewise influenced. In order to decide this question a group of experiments was performed on the effect of insulin coma on partially *conditioned rats*. Two groups of 9 rats were chosen for this experiment. Whereas in the main experiments described previously the rats were conditioned until they responded with the c.r. in nearly 100 per cent to the c.s. the "partially" conditioned rats were trained only up to the time when their behavior indicated that the conditioning process had begun. After various trials the following procedure was chosen. The rats were subjected for 2 to 5 days to not more than 25 reenforced c.s. (bell+shock) on each day. As soon as the animals jumped twice in succession in response to the c.s. before they were stimulated by the u.s. the training period was terminated. This occurred on the average, after 75 reenforced c.s. had been applied. Then the rats of one group were injected with 0.5 cc. saline on alternate days and rats of the experimental group were given insulin on two alternate days. On the following day 20 bell+shock stimuli were applied and then 10 non-reenforced c.s were given in order to ascertain the state of conditioning. In each of the two groups there was one animal (#8 of the control and #1 of the experimental group) in which the conditioning reaction was established with an unusually small number of combinations of c.s. and u.s. These animals

are therefore not utilized for averaging the results. Table 2 shows that when 20 reenforced stimuli are given following the injection of saline, the conditioned response was on the average 20 per cent, the individual values varying between 10 and 20 per cent. The experimental group injected with insulin was given on the average only 15 reenforced c.s., but in spite of this lesser

Table 2. Effect of Insulin Hypoglycemia on Partially Conditioned Rats

A. Controls

Animals	No. of Bell +Shock Applied for Partial Conditioning	Amount Saline Injected	No. of Bell +Shock Applied after 2nd Saline Inj.	Total No. Bell +Shock Applied	Conditioned Response on Testing
1	95	0.5 cc.	20	115	40%
2	77	0.5 cc.	20	97	30%
3	50	0.5 cc.	20	70	10%
4	53	0.5 cc.	20	73	20%
5	81	0.5 cc.	20	101	40%
6	110	0.5 cc.	20	130	0%
7	55	0.5 cc.	20	75	20%
8	31	0.5 cc.	3	34	90%*
9	82	0.5 cc.	20	102	0%
Average	75		20	95	20%

B. Insulin-Injected Rats

Animals	No. of Bell + Shock Applied for Partial Conditioning	Amount Insulin Injected in u/kilo Wt.	Effect of First Insulin Injection	Effect of Second Insulin Injection	No. of Bell + Shock Applied after Insulin Coma	Total No. of Bell + Shock Applied	Conditioned Response on Testing Per cent
1	22	10 u/kilo	Coma	Coma	7	29	100*
2	70	10 u/kilo	Depression	Coma	20	90	100
3	55	10 u/kilo	Coma	Coma	10	65	90
4	83	10 u/kilo	Coma	Coma	15	98	100
5	82	5 u/kilo	Depression	Coma	20	102	80
6	115	5 u/kilo	Depression	Coma	15	130	100
7	95	10 u/kilo	Depression	Coma	20	115	0
8	50	4 u/kilo	Coma	Coma	10	60	100
9	51	4 u/kilo	Coma	Coma	10	61	90
Average	75				15	90	82.5

* Not included in the average.

degree of training the c.r. amounted to 82.5 per cent. There was only one animal in the experimental group in which the conditioned response was not established.

It was mentioned earlier that we had in each group a rat which could be conditioned more easily than most animals and it was therefore, decided to exclude these animals from the average. Both rats showed clearly complete

conditioning in the control as well as in the experimental group. The fact that under control conditions an average of 95 combinations of c.s. and u.s. led in 8 animals to c.r. of only 20 per cent, whereas, 90 combined u.s. and c.s. led in the same number of animals to a c.r. in 82.5 per cent when the animals had been subjected to two insulin hypoglycemias shows clearly that the chronic effect of insulin hypoglycemia is not restricted to its action on intracentral inhibition but affects likewise those excitatory processes which are the basis of the establishment of c.r.

Discussion

In 1938 Rose, Tainton-Pottberg and Anderson (5) published a report on a single sheep in which after a conditioned reflex had been lost spontaneously this reflex was restored following a series of insulin shocks. The present report is a confirmation and extension of this work. It has been shown in normal rats that permanently inhibited conditioned reactions may be restored by means of insulin hypoglycemia. The effect of insulin was cumulative in as much as two to three comas were almost regularly effective whereas one coma frequently failed to alter the inhibited conditioned reactions. The duration of the effect was variable. Properly timed insulin comas resulted in a permanent recovery of the inhibited conditioned reactions. The behavior of the animals thus influenced was altered. They seemed to be more alert than prior to the "treatment." Moreover, the application of the non-reenforced c.s. to these "permanently recovered" animals failed to induce any inhibition as it regularly did in all animals not subjected to insulin hypoglycemia. When insulin coma failed to effect a permanent recovery of the c.r. a temporary restitution of the c.r. lasting several days was observed. It will be of interest to determine the factors underlying these individual differences.

It was mentioned earlier that the clinical syndrome resulting from the administration of insulin may be classified as depression, coma, or convulsion. Figure 5 gives a survey of all the results thus far obtained. It shows clearly that insulin coma is very effective whereas convulsions only infrequently restore inhibited c.r. As to hypoglycemic depression it may be said that it is mostly ineffective. One instance in which a significant increase in c.r. could be observed is that seen in Fig. 4 in which the c.r. were already partially disinhibited as a result of previously administered insulin comas. Such an effect has been observed once more subsequently under similar conditions.

The fact that insulin coma restored inhibited c.s. was interpreted as being due to disinhibition of c.r. This interpretation is justified on the basis of two sets of experiments. In the first it was shown that insulin coma failed to cause any measurable effects in rats which had not been conditioned previously, secondly, the reaction was specific in as much as the rats showed a positive response to the bell to which they had previously been conditioned, but failed to react to other sounds or visual stimuli.

However, the chronic effect of insulin hypoglycemia on the brain is not restricted to the elimination of intracentral inhibitions of the Pavlovian type. The experiments on partially conditioned rats show also that the excitatory processes which form the basis for the establishment of c.r. are facilitated. This is shown by the fact that insulin administered during the training period greatly enhances the formation of c.r. It is of interest to

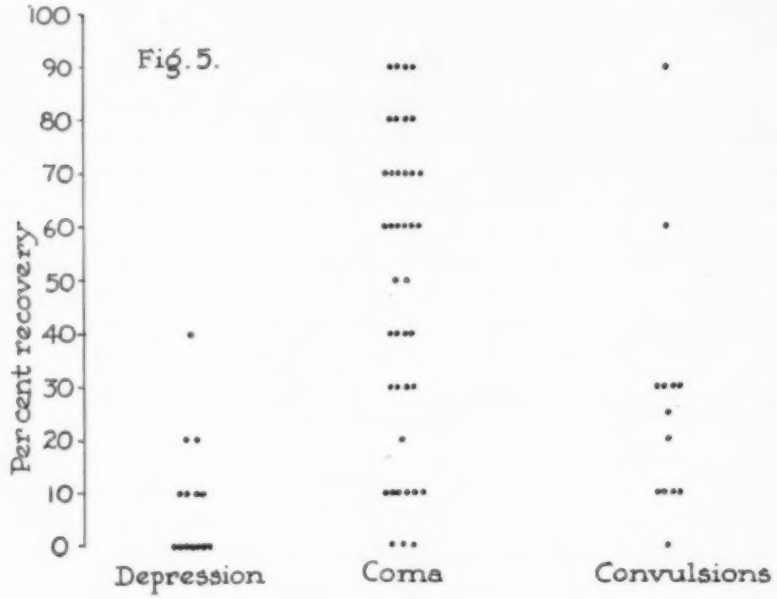

FIG. 5. Relative efficiency of insulin "depression," coma, and convulsions on the restitution of previously inhibited conditioned responses. The ordinate refers to the increase in the percentage of the conditioned response following administration of insulin.

point out that the chronic effects of insulin hypoglycemia are similar to those described by Kessler and Gellhorn (4) for metrazol and electroshock. Whether it is possible to induce a permanent recovery with these last two procedures has not yet been investigated, nor is it known whether these convulsive procedures enhance the *formation* of c.r. as has been shown for insulin in the present investigation.

The data presented in this paper as well as in the investigations of Kessler and Gellhorn (4) suggest that central sympathetic excitation which is common to all three procedures (1, 2, 3) may play an important part in the explanation of the altered behavior of man and animals subjected to various forms of "shock therapy." It will be the task of further studies to determine the neural structures which are necessray for the disinhibition of conditioned reactions by means of insulin coma or experimentally induced convulsions.

Summary and Conclusion

The chronic effect of insulin hypoglycemia on the central nervous system has been studied by means of conditioned reactions.

Normal rats which jump from a compartment A across a small partition to a compartment B in a response to an electric shock applied to the grid of the compartment (unconditioned response) are trained to react in a similar way in response to a conditioned stimulus (bell). This reaction is inhibited by lack of reenforcement. Under such conditions no spontaneous recovery occurs but insulin hypoglycemia restores the inhibited conditioned response. The action of insulin is cumulative and may lead to a permanent recovery. This effect of insulin depends upon the number of insulin administrations and the degree of hypoglycemia, coma being the most effective procedure. The reaction is specific since no positive responses are elicited when stimuli are used to which the animal had not been conditioned previously.

The chronic effects of insulin coma are not restricted to disinhibition of conditioned responses but influence also the excitatory processes which are the basis of conditioning. This is shown by the fact that a partial conditioning leading to an average of only 20 per cent positive responses in the control group causes 82 per cent conditioned responses in the experimental group subjected to two insulin hypoglycemias during the training period.

REFERENCES

1. Gellhorn, E. Effects of hypoglycemia on the central nervous system. *Arch. Neurol. Psychiat., Chicago*, 1938, *40:* 125–146.
2. Gellhorn, E. *Autonomic regulations. Their significance for physiology, psychology and neuropsychiatry.* New York, Interstate Publishers Inc., 1943. xii, 373 pp.
3. Gellhorn, E., and Kessler, M. Experimental investigations on the interaction of electroshock and insulin hypoglycemia. (In press.)
4. Kessler, M., and Gellhorn, E. The influence of electrically and chemically induced convulsions on conditioned reflexes. *Amer. J. Psychiat.* 1943. (In press.)
5. Rose, J. A., Tainton-Pottberg, A., and Anderson, D. D. Effects of insulin shock on behavior and conditioned reflex action in the well trained sheep. *Proc. Soc. exp. Biol. N. Y.*, 1938, *38:* 653–655.

CORTICAL LOCALIZATION OF SYMBOLIC PROCESSES IN THE RAT: II. EFFECT OF CORTICAL LESIONS UPON DELAYED ALTERNATION IN THE RAT

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DESPITE THE fact that the memory of maze performance in rats is not dependent upon any specific areas, recent experiments by Stellar, Morgan and Yarosh (20) have shown that a maze-performance, which excludes the use of external cues and requires symbolic processes, depends upon the anterior cortical areas remaining intact. This finding parallels the special role of the prefrontal areas of primates in controlling the mechanisms of recent memory and the synthesizing of a series of acts (9), and indicates that in the rat, as in primates, there may be specialized "association" areas.

As a further attack upon this problem we have selected another behavioral test, the delayed alternation, which requires the use of immediate memory, and have determined how it is affected by the removal of various cortical areas. An early study by Loucks (13) made use of a similar test and indicated that lesions of the "prefrontal" areas abolished ability to pass it, but his experiments failed to provide sufficient assurance that the same results might not also be obtained by lesions in other areas, and for this reason, as well as for verification, it seemed profitable to reinvestigate the problem.

METHODS

A number of years ago Hunter (8) devised the delayed reaction test of recent memory and the rat's performance on the test was subsequently studied in several experiments (16). A large number of trials was required to learn it, however, and at best rats never did very well; these two factors tended to discourage the use of the test in operative studies.

Recently Heathers (7) and others (2, 3) have described a phenomenon of spontaneous single alternation in rats, which involves the use of recent memory and serves the purpose of the present study. Rats are simply run on a T-maze in which both choices are rewarded, and once they have become familiar with the apparatus, they begin spontaneously to alternate, taking first the left alley, then the right, then the left, and so on. There has been considerable discussion as to why rats do this, but that need not concern us. The fact is that they do, and that in order to alternate at higher than a chance level they must remember what turn they took on the previous trial. Thus immediate memory, and not any habit learned over a series of trials, must be the basis of the alternation.

In the present study a T-maze of the following specifications was used: elevation, 30 inches above the floor; width of path, 2 inches; length of starting arm, 24 inches; length of each side arm, 18 inches. Purina dog chow, mixed with water to make a mash, was present at the ends of both arms at all times.

Twenty-four brown rats were begun in the experiment. They were first put on a 24-hour feeding schedule and allowed enough food (Purina mash) in each feeding period to bring them down in weight to a point where they would be healthy but highly motivated. This schedule was maintained for a week. Then on the eighth day they were placed, two at a time, on the maze and allowed to explore and eat for a half-hour. Finally, on the ninth day, regular tests of recent memory were run. They were placed on the start and allowed, without any forcing or interference, to choose either arm of the T to get food. They were allowed 15 seconds of eating, clocked on a stop-watch, before being taken from the arm and

placed at the start again. This 15-second interval insured that the recent memory being tested was of at least this duration. Eleven trials each day were given in order that ten opportunities to alternate be provided. Preoperative tests consisted of six such days, thus allowing a maximum of 60 alternations. At the end of this time the scores of all animals were examined and those animals were discarded which had not alternated 36 or more times, that is, 60 per cent of the possible number, and that percentage which is significantly different, statistically, from 50 per cent. Six of the 24 animals did not reach this criterion and were dropped from further study. The remaining 18 were then subjected to operation.

The operative technique was a modification of the method of thermocoagulation previously described by Finley (4). The operative instrument was an electric soldering iron whose tip was cut down to present a round flat surface 3 mm. in diameter. This tip was applied to the surface of the skull bones after an incision had been made, under ether anesthesia, in the skin and cranial musculature. By varying the position on the top of the skull and by regulating the time and temperature of the application according to calibrations previously worked out on trial animals, lesions could be made in the cortex in almost any desired position and of an appropriate size. The resulting lesions were almost perfectly circular in shape, were clearly demarcated from surrounding intact tissue, and were free from the mechanical distortion which often accompanies the use of a thermocautery applied directly to the cortex. In addition to these features, the technique has the advantage of producing much less operative shock than methods requiring trephining of the skull.

The animals were kept upon the 24-hour feeding schedule over the operative and recovery period so that there would be no changes in motivation to disturb postoperative testing. This was begun 7 to 10 days after the operation, and like the preoperative tests, was conducted for 6 days at 11 trials per day. In a few instances animals were run 21 trials per day, thus allowing 20, rather than 10, alterations, but analysis of the records shows that this had no effect upon the final results. One animal died three days after operation and thus did not enter upon postoperative tests.

Upon conclusion of these tests, animals were kept alive for one month or more after the operation before removing their brains. This was done immediately upon death under ether and the brains were placed in 95 per cent alcohol for two weeks. After fixing and before blocking, the position and extent of the lesions were determined in each case by using dividers and drawing the lesions upon standard diagrams prepared by Lashley (11). In view of the fact that the operative technique produced cleanly demarcated lesions without distortion in the shape of the brain, this method of reconstruction was just as accurate as that done from histological sections, except that damage to subcortical nuclei could not be seen in this way. Previous studies of the effects of the time and temperature of the thermocoagulation, however, had already indicated that no subcortical damage, except in case of infection, was to be expected.

Results

The pre- and postoperative performances on the delayed alternation tests, as well as a specification of the position and extent of the lesions in each case, are summarized in Table 1. In order to make the results readily understood, the rats have been arranged and numbered approximately in the order they were affected by the operation. Rats 1 and 2, for example, were most affected and rats 16 and 17 least affected by the operation.

Table 1 gives the pre- and postoperative performance of each rat and Fig. 1 shows the position and extent of each lesion as ascertained after removal and fixing of the brain. It will be seen in Table 1 that there was some falling off in postoperative performance of every single animal irrespective of the kind of cortical lesion inflicted. In many cases the reduction was slight, yet there is no exception to the rule. This fact may indicate one of two conclusions: either that there is a general tendency for spontaneous alternation to decrease as animals have run more and more trials or that a cortical injury of any kind reduces somewhat the amount of alternation.

Experimental evidence available at the present time does not allow us to decide definitely between these possibilities, although there is some indication (7) that single alternation tends to decline somewhat with time.

Since the purpose of this study is to determine whether or not capacity for single alternation is "localized," the over-all decrement in performance is important only because some allowance should be made for it in considering how seriously operations affected the alternation. Taking this tendency into consideration, the cases may be divided into three groups: Group A, consisting of 6 animals (Nos. 1–6), in which alternation was almost com-

Table 1. Percentage of single alternation in 60 preoperative and 60 postoperative trials

Group A Prefrontal Lesions			Group B Various Lesions			Group C Occipital Lesions		
Rat No.	Pre-	Post-	Rat No.	Pre-	Post-	Rat No.	Pre-	Post-
1	80	10	7	75	25	12	67	57
2	83	13	8	70	28	13	70	60
3	62	0	9	70	41	14	75	65
4	77	15	10	78	55	15	68	62
5	68	10	11	82	70	16	62	58
6	77	19				17	62	58

pletely abolished and was, in any case, reduced below the 50 per cent level expected of animals making their choices completely at random; Group B, made up of 5 animals (Nos. 7–11), in which the operation was of less consequence; and Group C, comprising 6 animals (Nos. 12–17), whose postoperative performance was not significantly reduced. These may be designated the affected (A), doubtful (B), and unaffected (C) groups respectively.

For purposes of exposition, we may divide the rat's cortex into four general areas, delimited by the numbered levels on the prepared diagrams, and assign them names comparable to roughly corresponding areas in the primates: prefrontal areas, levels 1 to 4; frontal areas, levels 4 to 9; parietal areas, levels 9 to 13; and occipital areas, levels 13 to 18.

The lesions sustained by animals of Group A are shown in the left hand column of Fig. 1. In every single case, it is to be noted, the prefrontal quarter of the cortex is significantly implicated. In the two most affected cases, the lesion is squarely at the anterior poles. In three other less affected cases, some of the more posterior frontal ("motor") areas are involved, but all of them overlap the prefrontal areas somewhat.

The unaffected animals in Group C all have lesions in the posterior cortex, in most cases overlapping the visual areas, and safely out of the central dorsal region subserving motor functions. They present positive proof that the occipital areas are not necessary to the functions involved in spontaneous single alternation.

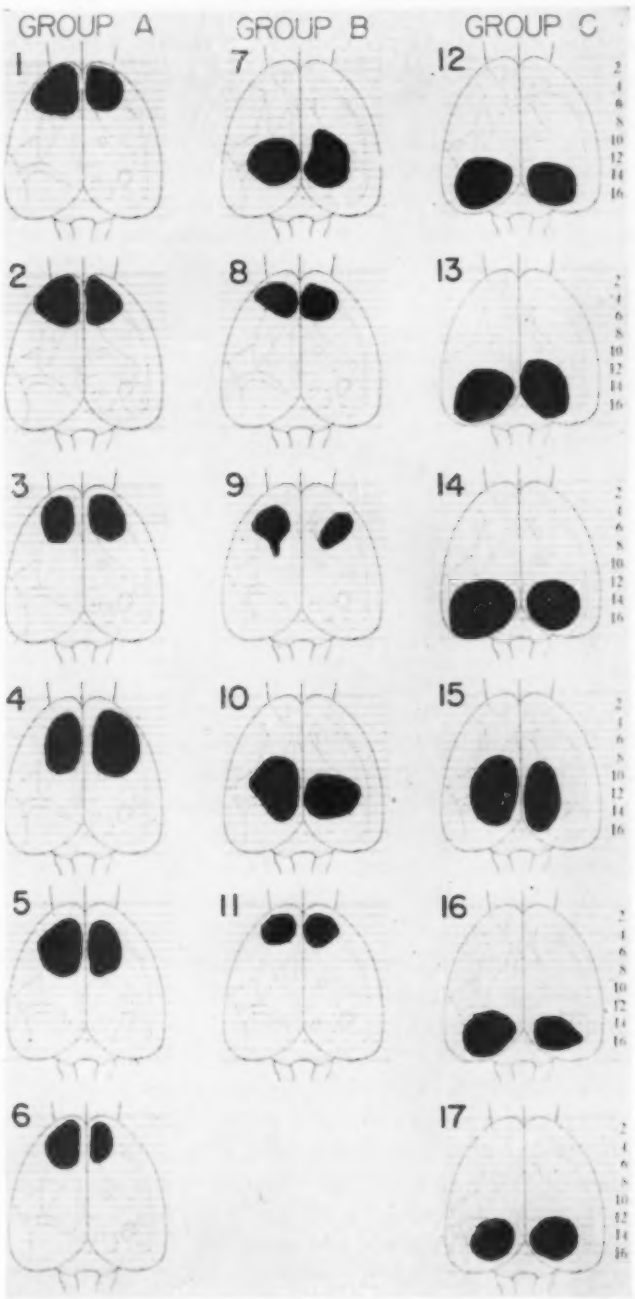

FIG. 1. Maps of the lesions sustained by the 17 rats tested on delayed alternation. Group A (Nos. 1–6) consists of animals whose behavior was greatly affected by operation; Group B (Nos. 7–11) of animals with small or doubtful postoperative disturbances; and Group C (Nos. 12–17) of rats not affected significantly by operation.

The picture presented by Groups A and C is perfectly clear and consistent, and it calls for the "localization" of the capacity of delayed single alternation in the anterior cortical areas. Group B, the doubtful animals, presents some difficulties, however. One case in it, No. 8, falls in line with the other results, for it shows a postoperative decrement which is probably significant and its lesion is largely prefrontal. Case No. 9, however, has little involvement of the prefrontal region, yet it shows a moderate, but significant, postoperative loss. From it we might suppose that the frontal motor areas also function in recent memory. Case No. 7, on the other hand, shows a loss almost as great as those in Group A; and its lesion is in the parietal areas. Case No. 10 is like it in respect of position of lesion, but the loss in behavioral performance which it sustained, though probably significant, is less definite. One animal in Group C (No. 15), on the other hand, has almost the same type of lesion as Nos. 7 and 10, and it shows no postoperative deficit. Thus, the parietal lesions do not give consistent behavioral effects.

Case No. 11, in Group B, is the last to be considered. It shows hardly any postoperative loss, but its lesion is prefrontal. It is, in fact, the only one of 9 cases with prefrontal involvement that was not significantly impaired by the operation. It may be pointed out, however, that the size of its lesion was small compared to the other prefrontal cases, and an inspection of the depth of the lesion, in addition, indicates that it probably does not penetrate the entire cortex. Assuming, then, that a reasonably large area of the prefrontal cortex is involved in the recent memory function, it might be said that this case left enough of the critical area intact to allow it to function without significant impairment.

Discussion

Making allowances for the superficial lesion of case No. 11, two definite conclusions can be drawn from the present results: destruction of the prefrontal areas abolishes passable performance in the delayed alternation test; and lesions of the occipital areas have no significant effect upon such performance.

The role, however, of the "motor" and parietal areas in this performance is left in doubt. The one case of a motor lesion free of implication of the anterior areas showed a similar, though smaller, reduction in score. Parietal lesions, on the other hand, did not give a consistent picture; one produced considerable impairment, another slight impairment, and still a third none at all. In view of the motor and kinesthesis functions subserved by these areas between the anterior and occipital poles, it is probably to be expected that their extirpation might have some effect upon the performance, but the present results do not indicate that such a possible role is primary. It is probably best to conclude, pending future experimentation, that the parietal areas do not make a primary contribution to the performance but may have some importance in it.

In the previous paper by Stellar, Morgan and Yarosh (20) in which the

distance-discrimination test of symbolic processes was studied, it was pointed out that the critical areas for symbolic functions in the rat, although not well defined, tend to lie in the fields of projection of the anterior and medial divisions of the ventral thalamic nucleus. The same statement can be made from the present findings. It should be noted, however, that the projections of the dorsomedial thalamic nucleus also project to this region of the cortex (11). In primates this nucleus projects to areas 9, 10 and 12 of the prefrontal lobe (21) and degeneration in it is associated with the psychological phenomena of prefrontal lobotomy in human individuals (5). It is logical, therefore, to suppose that the effects upon recent memory which are here reported depend upon the projection field of the dorsomedial nucleus. There are, unfortunately, no comprehensive studies of the exact areas served in the rat by this nucleus, but only observations based upon a few cases (11). Taking these observations, however, and also the result of this and the previous study (20), there is now good reason to believe that there exists in the rat a prefrontal area which is roughly comparable anatomically and in the behavioral functions which it serves to the prefrontal "association" areas of man.

Lashley's earlier studies (12) of cortical areas involved in maze learning yielded no evidences of functional localization in the rat's cortex. The number of psychological elements concerned in maze learning, however, is so great that this might be true despite relatively discrete functional localization of different specific capacities. Studies, moreover, in which less complex behavioral performances have been considered, have tended on the whole to indicate a limited localization of functions. The striate area is necessary for visual pattern discriminations (10), a temporal area for certain auditory discriminations (6, 17, 22), a dorsal "motor" area for hopping and placing reactions (1), a more frontal area for motor coordination (14) and for hand preference (18), and another dorsolateral area for tactual discrimination (19). Now, to this list should be added a prefrontal "association" area for recent memory.

It may turn out upon further study that the loss following removal of this prefrontal area is not so much of recent memory as it is of the ability to maintain a set in the face of distraction. That at any rate has been the case in the primate studies. Prefrontal lobectomized monkeys are capable of solving delayed reaction tests if precautions are taken to see that the animal is not distracted during the delay (15), whereas under ordinary circumstances the capacity is abolished by prefrontal lobectomy (9). In our experiments animals were open to considerable distraction during the delay; they were picked up from the feeding dish, moved to a chair where they remained during the delay while the experimenter was moving around and recording the results of the previous trial, and then finally picked up and set down on the start again. Thus our experiments might very well find recent memory disturbed by the operations when the more fundamental factor is ability to withstand distracting influences. In further experiments the attempt can be made to separate these two factors from each other.

Summary

The experiment was designed to determine whether or not there are limited areas in the anterior cortex of the rat which subserve symbolic processes, and in particular recent memory, as has been found to be the case in primates.

In a T-maze in which rats spontaneously alternate, 17 rats were run for 60 trials each, pre- and postoperatively. Between each trial there was an interval of 15 seconds and single alternation significantly greater than a chance level of 50 per cent was taken as an index of the use of recent memory. Following completion of postoperative tests brains were removed and the size and location of the lesions reconstructed upon standard diagrams. The majority of the lesions were in either the prefrontal or the occipital areas. Animals with the prefrontal lesions, except for one with a small lesion, lost their ability to use recent memory, whereas those with occipital lesions showed little difference in performance. Too few 'pure' motor lesions were available to determine whether these also affected the recent memory. Parietal lesions, on the other hand, gave inconsistent results.

Some "localization" of recent memory in the anterior areas of the rat is definitely indicated, although more work is required to tell us how precise it may be. Considering the other cases of functional localization of specific functions established in recent years, the rat's cortex appears not to be so poorly differentiated as earlier maze studies lead us to believe.

REFERENCES

1. Brooks, C. M. Studies on the cerebral cortex. II. Localized representation of hopping and placing reactions in the rat. *Amer. J. Physiol.*, 1933, *105:* 162–171.
2. Dennis, W. Spontaneous alternation in rats as an indicator of the persistence of stimulus effects. *J. comp. Psychol.*, 1939, *28:* 305–312.
3. Dennis, W., and Sollenberger, R. T. Negative adaptation in the maze exploration of albino rats. *J. comp. Psychol.*, 1934, *18:* 197–206.
4. Finley, C. B. Equivalent losses in accuracy of response after central and after peripheral sense deprivation. *J. comp. Neurol.*, 1941, *74:* 203–237.
5. Freeman, W., and Watts, J. W. *Psychosurgery*. Springfield, Ill., C. C Thomas, 1942, xii, 337 pp.
6. French, R. L. The function of the cerebral cortex of the rat in the discrimination of simple auditory rhythms. *J. comp. Psychol.*, 1942, *33:* 1–32.
7. Heathers, G. L. The avoidance of repetition of a maze reaction in the rat as a function of the time interval between trials. *J. Psychol.*, 1940, *10:* 359–380.
8. Hunter, W. S. The delayed reaction in animals and children. *Behav. Monogr.*, 1913, *2:* No. 1, v, 86 pp.
9. Jacobsen, C. F. Studies of cerebral function in primates. I. The functions of the frontal association areas in monkeys. *Comp. Psychol. Monogr.*, 1936, *13:* No. 3, 60 pp.
10. Lashley, K. S. The mechanism of vision. IV. The cerebral areas necessary for pattern vision of the rat. *J. comp. Neurol.*, 1931, *53:* 419–478.
11. Lashley, K. S. Thalamo-cortical connections of the rat's brain. *J. comp. Neurol.*, 1941, *75:* 67–122.
12. Lashley, K. S. *Brain mechanisms and intelligence*. Chicago, Univ. Chicago Press, 1929, 186 pp.
13. Loucks, R. B. Efficacy of the rat's motor cortex in delayed alternation. *J. comp. Neurol.*, 1931, *53:* 511–567.
14. Maier, N. R. F. The cortical area concerned with coordinated walking in the rat. *J. comp. Neurol.*, 1935, *61:* 395–405.

15. MALMO, R. B. Interference factors in delayed response in monkeys after removal of frontal lobes. *J. Neurophysiol.*, 1942, *5:* 295–308.
16. MUNN, N. L. *An introduction to animal psychology*. New York, Houghton Mifflin, 1933, (see pp. 353–373).
17. PENNINGTON, L. A. The effects of cortical destruction upon responses to tones. *J. comp. Neurol.*, 1941, *74:* 169–191.
18. PETERSON, G. M. Mechanisms of handedness in the rat. *Comp. Psychol. Monogr.*, 1934, *9:* (No. 6), 1–67.
19. SMITH, D. E. Cerebral localization in somaesthetic discrimination in the rat. *J. comp. Psychol.*, 1939, *28:* 161–188.
20. STELLAR, E., MORGAN, C. T., and YAROSH, M. Cortical localization of symbolic processes in the rat. *J. comp. Psychol.*, 1942, *34:* 107–126.
21. WALKER, A. E. *The primate thalamus*. Chicago, Univ. Chicago Press, 1938, xxvi, 325 pp.
22. WILEY, L. E. Function of the brain in audition. *J. comp. Neurol.*, 1932, *54:* 105–135.

MOTOR RESPONSE TO STIMULATION OF CEREBRAL CORTEX IN ABSENCE OF AREAS 4 AND 6 (*MACACA MULATTA*)*

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PREVIOUS experiments have established two facts with regard to the influence of the cerebral cortex on motor performance of monkeys: (i) Ablation of the motor areas (areas 4 and 6 of Brodmann) in infancy has less effect than removal of the same areas from older animals; (ii) removal of the frontal areas (areas 8–12) or of the postcentral gyrus (areas 3, 1, 2) from normal animals does not cause paresis, but, if areas 4 and 6 have been previously removed in infancy, subsequent extirpation of either frontal or postcentral regions is followed by a marked increase in motor deficit (2).

Some reorganization of function of these "non-motor" regions must thus occur after areas 4 and 6 have been removed in infancy. Since these altered areas might then become more excitable to cortical stimulation, the cortices of five nearly adult monkeys have herewith been stimulated following motor ablations made at a much earlier date. Four of these animals had been operated upon in infancy and the excitability of their cortices explored more than two years thereafter. The fifth, used as control, was nearly mature and had been operated upon five months previously. In all, apparently maximal recovery of function had taken place some time before the terminal experiment.

METHOD

The five animals were all *Macaca mulatta*. The four operated upon in infancy were born in the colony and of known age. The fifth was much older, a nearly mature male weighing 4.2 kg. All cortical ablations were made by the same individual and with the same technique. Areas 4 and 6 were totally removed from one or both hemispheres of four monkeys. The lesion extended to the depth of the cingulate gyrus on the medial aspect and through the face area laterally. It is probable that all of area 6b was not removed in every case, since its margins are indefinite and vary with the shape of the arcuate sulcus. The extirpation was taken to the depth of the central sulcus. The fifth animal had bilateral extirpation of areas 4, 3-1-2 comprising the pre- and postcentral gyri. Area 6 remained intact.

It was necessary in the case of the control animal to use a preparation with a unilateral ablation because older animals with bilateral ablations are so severely paralyzed that they are difficult to keep alive, and, it was felt that a preparation of long-standing to insure maximal recovery was essential for comparison with those of infants showing maximal recovery after bilateral ablations. At the time of sacrifice, the cortices were exposed under dial anesthesia (0.6 cc. per kg. given one-half intraperitoneally and one-half intramuscularly). Stimulation was with bipolar electrodes by a current controlled for wave form, frequency and voltage. After removal of brain at autopsy, sections were made and stained by Nissl technique so that the extent of the lesions might be verified histologically.

* Aided by grants from the Friedsam Foundation (Child Neurology Research), and the Macy Foundation.

Experimental Data

A summary of the findings follows. The results of stimulation of cerebral cortices are prefaced by a brief description of the motor status of each animal just prior to the terminal experiment.

Experiment I (R.S. 8) July 29, 1942. A large, nearly mature male, wt. 4.2 kg. Lt. areas 4 and 6 removed Feb. 20, 1942.

During the five months since the first operation the right motor paresis had become slight and had not improved after the first two or three months. The paresis was characteristic of that seen in all animals following similar lesions. There was weakness and awkwardness in the right extremities particularly in the hand. However, the animal was able to

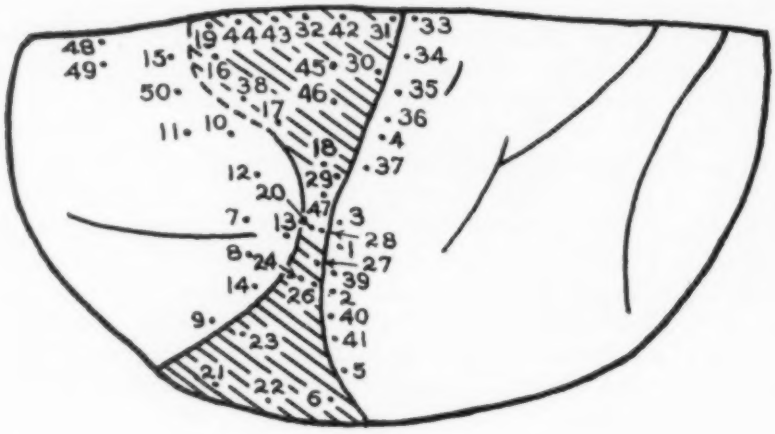

Fig. 1

climb using all four extremities and prehension was present in the right fingers although of a simple order as compared to that of the normal left hand. Tendon reflexes were increased on the right side; there was slight atrophy of the limb muscles of this side and slightly increased resistance to passive manipulation.

Stimulation: In Fig. 1, the points stimulated on and about the scar of the left areas 4 and 6 ablation are designated numerically. *No* response to any stimulus was obtained from any point except from the following:

Point	*Response*
7.	Dilatation of lt. pupil
8.	Eyes turned rt.
9.	Convergence of eyes
10.	Lid blinking, convergence
12.	Blinking followed by pupil dil. and lachrymation from rt. eye
13.	Rt. eye turned up, lt. down, long latency
38.	Eyes turned rt., blinking, movement of rt. arm.
48.	Blinking
49.	Blinking
50.	Eyes turned rt. arm movement

Summary: Stimulation elicited appropriate movements of eyes, pupils and lids from area 8. Diffuse arm movements were also obtained from what was thought to be area 8 but may have been borderline area 6. The entire postcentral gyrus and the scar tissue were inexcitable.

Experiment 2 (I 37) Jan. 19, 1942. A male, 3 years, weight 2.4 kg. Born Apr. 27, 1939;

ablation of left areas 4 and 6 Sept. 30, 1939; ablation of right areas 4 and 6 Oct. 30, 1939; cord transection at Th. 5 Jan. 2, 1942.

Following the cortical ablations during the 6th and 7th months of life this animal recovered sufficient motor skill so that it was able to feed and carry on ordinary cage activities. It had, however, marked scissors gait in the hind legs and used fingers poorly in voluntary prehension. Resistance to passive manipulation was moderately increased and tendon reflexes were markedly hyperactive. For another purpose the cord had been transected in the mid-thoracic region prior to terminal experiment. Subsequent stimulation on the cortex was consequently valid only in those regions supplying upper extremities or face.

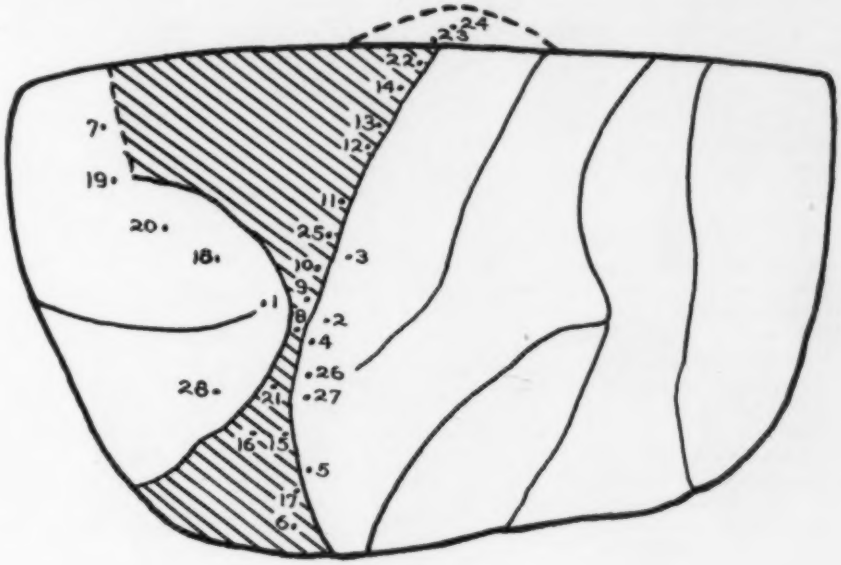

FIG. 2

Stimulation: In Fig. 2, the points stimulated on the left cortex are shown. *No* response was obtained at any point except the following:

Point	*Response*
8.	Tongue withdrawn
9.	Tongue withdrawn, movement of lower lip
10.	Tongue, shoulder and hand movement
12.	Tongue, shoulder and arm
13.	Tongue withdrawn, fingers flexed
14.	Fingers, upper lip
15.	Tongue
16.	Protrusion of tongue
19.	Eyes turned rt.
21.	Tongue, torsion
22.	Upper lip
25.	Tongue

Summary: Normal characteristic movements of lids and eyes resulted from stimulation of area 8. Discrete rapid movements of the lip were obtained with a stimulus of the type usually effective in area 4, from points 14 and 22. Slow generalized movements of the fingers appeared from stimulation of points 12 and 13. They were usually accompanied by tongue and lip movements. There were no discrete finger movements but instead slow diffuse flexion of fingers and wrist, with occasional spread to forearm. Movements of the tongue and lip were obtained from points 8, 9, and 10 easily and quickly and with low volt-

ages. Higher voltages caused the same movement at longer latency from any spot in the entire scar. This was obviously due to spread of current via the scar tissue. Movements of arm and face were elicited from the entire length of the posterior lip of the central sulcus.

Experiment 3 (I 50) July 17, 1942. A male, 2 years, weight 2.1 kg. Born Jan. 8, 1940. Bilateral ablation of areas 4 and 6 Jan. 30, 1940.

Simultaneous bilateral removal of areas 4 and 6 produced a deficit in this infant which was similar in kind but slightly more severe than that of the animal in Expt. 2. There was marked crossing of the hind limbs in walking, increased resistance to passive manipulation and active tendon reflexes. Voluntary prehension was almost impossible, but climbing and clinging were well executed.

Stimulation: In Fig. 3, A and B, the points stimulated on the left and right cortex respectively are indicated numerically. No response was obtained except from the following points:

Lt. Cortex	
Point	*Response*
5.	Arm, wrist extension
7.	Arm, more marked
8.	Elbow
9.	Diffuse arm
10.	Elbow and wrist, eversion
11.	Elbow and wrist, eversion
14.	Deviation of eyes rt.
15.	Convergence of eyes
16.	Lids twitch, pupils dilate
17.	Lids, followed by arm
18.	Shoulder, after long latency
19.	Wrist and elbow
21.	Extension of wrist
22.	Upper arm and face
23.	Eyes turned to lt.
25.	Lt. eye turned rt.
27.	Rt. shoulder
28.	Upper lip, slight
30.	Extension of wrist, with after-discharge
31.	Wrist extension, followed by elbow
32.	Upper lip, then arm
33.	Rt. eye to lt.
34.	Convergence, pupils dilate spread to lower jaw
35.	Upper lip
37.	Diffuse shoulder
38.	Upper lip, shoulder, elbow
41.	Wrist extension
43.	Wrist
44.	Wrist and elbow
46.	Pronation
47.	Flexion of index finger, then wrist

Rt. Cortex	
Point	*Response*
1.	Face
2.	Face
3.	Face
5.	Face
6.	Face
8.	Face
9.	Face
10.	Lids opened
13.	Eyes to lt. slight
15.	Eyes to lt.
16.	Eyes to lt. slowly
17.	Eyes to rt.
18.	Eyes to lt. lids open, pupils dilate
19.	Lids open, then close
20.	Adduction and flexion of forearms
25.	Vertical nystagmus, lachrymation
26.	Lids open, eyes move down, homolateral arm moved
27.	Convergence of eyes, flexion of rt. extension of lt. arm
31.	Extension of wrist
32.	Extension of wrist
33.	Internal rotation, shoulder
34.	Pronation, wrist
37.	Abduction, thumb
47.	Extension of wrist, then thumb
51.	Eyes opened, chewing
52.	Extension wrist, then supination
53.	Abduction, shoulder
59.	Abduction, wrist 60
60.	Extension and eversion, wrist
61.	Shoulder and elbow
63.	Face and neck
64.	Thumb adducted
66.	Eversion and flexion wrist, some eye movements

Summary: From the intact area 8 characteristic eye movements were elicited. From stimulation the depth of the rostral portion of the scar, diffuse slight movements which were of the type elicited from area 6 of an intact hemisphere, appeared. Movements of hands, wrists and forearms were obtained from the posterior lip of the central sulcus in areas 3 and 1. Strychnine placed in an excitable point within the scar did not increase excitability. This was taken to indicate that excitability at this point was due to spread on scar tissue and not to active cells within this area.

Experiment 4 (I 60) July 18, 1942. Male, 2 years, weight 2.3 kg. Born Apr. 17, 1940. Ablation of left areas 4 and 6, May 6, 1940; of right areas 4 and 6 Sept. 20, 1940.

The motor deficit of this animal during the year before the terminal experiment was stationary and of the same order as that of the animal in Expt. 3 but not as extreme. This last was because the ablations were seriatim and not simultaneous. There was moder-

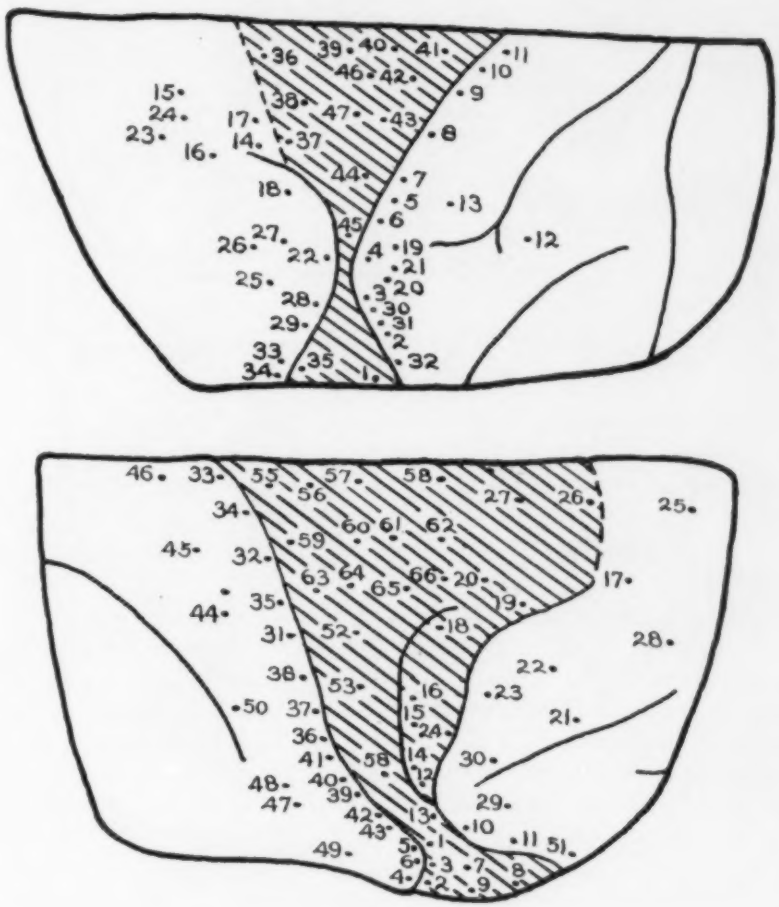

FIG. 3

ate spasticity and marked crossing of the hind legs in walking. The forelimbs were everted, the hands and fingers flared widely. Fingers were used awkwardly for voluntary prehension.

Stimulation: Figure 4 shows the points stimulated in both left and right sides. Those from which responses were obtained were as follows:

Lt. Cortex		*Rt. Cortex*	
Point	*Response*	*Point*	*Response*
2.	Face	10.	Hip and shoulder
3.	Face	11.	Leg, tail and shoulder
8.	Face	12.	Extension of forearm
9.	Face, then wrist and extension, finger	15.	Extension, wrist and fingers
10.	Extension, fingers	16.	Flexion, elbow, then hand
11.	Thumb extension, then wrist	17.	Eversion forearm

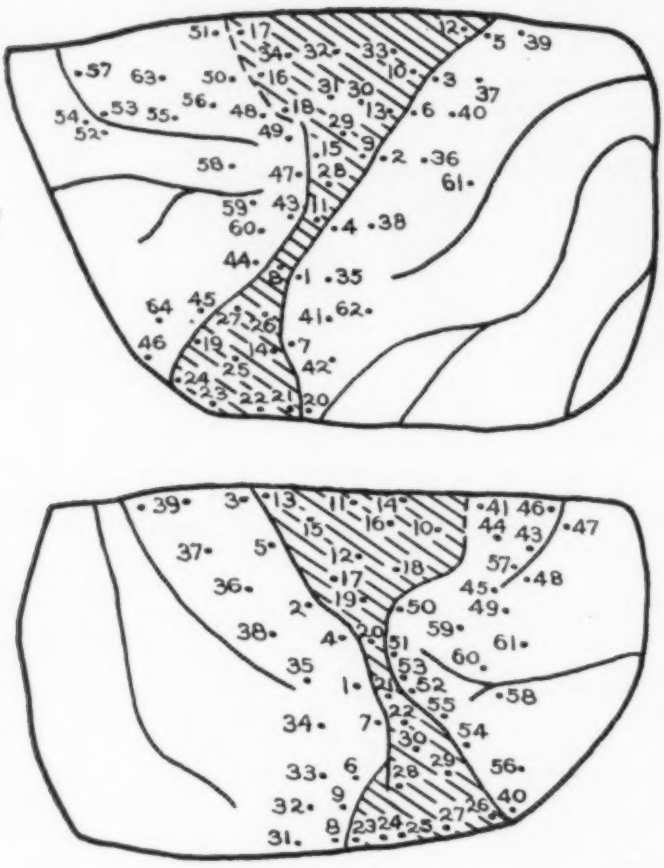

FIG. 4

13. Rt. upper lip
18. Rt. upper lip
28. Supination of wrist
29. Adduction at shoulder
30. Extension of wrist, finger, then lip
31. Lip followed by hand
32. Lip
33. Extension of wrist
34. Lip, slight
43. Eyes to rt.
44. Eyes to rt.
51. Dil. of pupils
58. Eyes moved to center
63. Eyes rt. and up; lachrymation
18. Inward rotation, shoulder
19. Inward rotation, shoulder
20. Inward rotation, shoulder
41. Eyes lt.
42. Eyes lt.
43. Eyes lt. and up, pupils dil.
44. Eyes down, rt. pupils dil.
45. Convergence
46. Lids blink
50. Convergence of eyes, slight
56. Eyes to lt.
57. Eyes to rt.
59. Eyes lt. pupils dil.
60. Eyes to lt.

Summary: Area 8 was intact and gave characteristic responses. Within the scar, movement of wrist and hand were obtained from one area. These were diffuse and irregular. Areas 3 and 1 on the posterior lip of the central sulcus were excitable. All movements were in the arms and face and were diffuse and generalized. In this animal as in the preceding three, the voltage threshold of stimulus producing movement was high, far higher than in

the normal cortex, both in the scar tissue and the surrounding regions with the exception of area 8. This latter area normally requires a stimulus of longer pulse form and longer duration than do areas 6 or 4. There was no detectable alteration of the excitability of area 8 in the present instances.

Experiment 5 (I 24). Mar. 16, 1942. Male, weight 3.4 kg. Born Apr. 24, 1938. Ablation of left areas 4, 3, 1 and 2; Dec. 9, 1938; ablation of right areas 4, 3, 1 and 2; cord transection at Th. 5, Feb. 25, 1942.

After the bilateral cortical ablations and preceding the cord transection (which was done for another purpose) this animal had definitely deficient motor performance, although it was not nearly as disabled as those of the preceding three experiments. It was able to use its hands accurately for feeding, although fine movements of prehension were impossible. It ran and jumped awkwardly. Placing and hopping reactions were absent on both sides. It was moderately spastic. To save space the map and list of points are omitted here and the findings summarized below.

Summary: On both hemispheres, responses to stimulation of area 8 were normal, as they had been in the preceding experiments. Caudal to the scar from areas 5 and 7, no response to stimulation was obtained in either hemisphere. Movements of face and tongue were elicited from each side lateral to the scar of area 4. There was evident spread to this region from stimulation of the scar. Stimulation of area 6 produced movements of arms and fingers characteristic of those usually produced from an intact hemisphere. No suppression was produced in what might have been area 4-s. It was therefore assumed that this area had actually been destroyed at operation.

Discussion

The result of cortical stimulation in the above five instances is sufficiently consistent to be considered valid and therefore merit discussion.

Stimulation of adult animal (control): Stimulation of the cortical tissue about the scar in the older animal produced a characteristic and normal response of eyes and lids from the intact area 8, and of the arm from what was possibly a remnant of the rostral part of area 6, but nothing from either the scar tissue or the postcentral gyrus. These findings confirm many chance observations made previously during stimulations of a large number of cortices of adult or nearly adult monkeys after cortical ablations made, usually, just prior to the stimulation, with one exception, namely, that the post-central gyrus is sometimes but not always excitable. In an intact cortex of *Macaca mulatta* under proper dial anesthesia as were these animals, stimulation of the posterior lip of the central sulcus will usually produce movement as will stimulation much farther caudally in the parietal lobe.

Stimulation of cerebral cortices of animals operated in infancy: Stimulation of these cortices produced much more movement than did that of the older animal. This might be expected and is consistent with the much greater adequacy of motor function of the animals operated on at early age. Movements elicited from all four "infants" had the same characteristics: (i) In all five animals the excitability characteristics of area 8 were alike and were the properties normally found on stimulation of area 8 in an intact cortex. (ii) Stimulation elsewhere always required a stimulus of much greater intensity and often of either longer duration or of longer pulse form than normal. (iii) Responses were always diffuse and poorly localized. There was obvious conduction of stimulus along scar tissue such that a given response (*i.e.* tongue, Expt. 2) could be produced from a wide area of scar tissue. This spread of stimulus seemed greater than that which is found in any scar

tissue and furthermore, the same property appeared in all other excitable areas with the exception of area 8, and, in one instance (Expt. 5), area 6. The spread of excitability did not confirm in time relations or in geographical distribution to that which would be expected from facilitation. It appeared directly related to the diffuseness of response to all stimuli. It may be related also to the lack of discreteness in movements during life. (iv) In every instance the excitable regions were those bordering on the scar and those which, in the intact cortex also, are excitable.

A great number of the movements were of tongue, lip and face and were directly related to the more complex movements produced by stimulation of area 6b. Others appeared on stimulation of the post-central gyrus, but were more diffuse and required higher threshold for response than were elicited from the same region of an intact cortex. In the three animals in which the lower extremities were responsive this obtained in the leg area as well as in arm and face. Movements characteristic of stimulation of area 6 were produced from the animal in which area 6 had not been removed at operation (Expt. 5) and did not differ in any way from those produced from the same area of an intact cortex. In the other animals occasional diffuse arm, neck and shoulder movements occurred associated with movements of the eyes. These likewise can be produced at the junction of areas 6 and 8 in the intact cortex.

Causes of differences: Stimulation of the control cortex and those of the four animals operated in infancy demonstrated marked differences between the two types since much more movement was produced from tissue around the scar in the latter than in the control. This would be attributed to any one of several causes.

(i) The most obvious possibility is that the extirpations of the four animals operated on in infancy were less complete than that of the control. This is unlikely for the following reasons: (a), grossly, at autopsy, the scar and the surrounding tissues were the same in extent on all five animals; (b), examination of the histological sections of all five preparations showed about the same extent of each lesion. In every instance there were large motor, possibly Betz cells, on the posterior lip of the central sulcus, on the inferior lip of the cingulate sulcus and, in a few instances, in the anterior lip of the arcuate sulcus. They were not different in size nor in number from the cells of the same type normally found in these sites in the macaque cortex. There was no other indication of any histological difference between the cortex of the animal operated on late in life and those operated on in infancy; (c), previous findings on a large number of monkeys operated on either in infancy or later have been the same, namely that there is always a great deal better motor function in the animals operated on in infancy than in the adults, and that this is without any demonstrable difference in anatomy or histology.

(ii) The second possibility is that there is a change in the excitability of the remaining tissue normally concerned with motor function. From the

present data this seems to be the most probable, for, in the present four cases the tissue surrounding areas 4 and 6, or the scar left by their ablation, was more reactive to electrical stimulation than is that of the intact cortex. However, no excitable region or regions were found in these cortices which would not be excitable in the intact cortex also. The difference was one only of degree. The most marked change in those animals operated on in infancy was the great spread of response from stimulation of one point, and, along with this, the similarity of the response obtained from a number of adjacent or fairly widely separated points.

The above explanation of these phenomena fits both the excitability characteristics of the cortices, and the functional characteristics of motor performance in these animals. It is one that has been offered before and has been described previously in the literature, namely that there is a reorganization of remaining tissue within a partially damaged functional unit (1), that of the motor system in this case. There is, at present, no evidence for anatomical reorganization accompanying this although, in the presence of functional reorganization, some anatomical change permitting wider spread of impulses through synaptic connections is still possible.

Summary

1. Stimulation of the cerebral cortices of *Macaca mulatta* from which areas 4 and 6 had previously been removed has revealed marked differences in excitability of the cortex from which the motor areas has been removed in infancy as compared to that from which motor areas have been removed later in life.

2. The cortex of the animal with motor areas removed in infancy has greater excitability in the regions surrounding areas 4 and 6, namely the posterior lip of the central sulcus, areas 6b and 6a than has either the normal macaque cortex or that of the animal from which the motor areas have been removed at a later age.

3. Movements elicited from these regions are more diffuse and require a higher threshold stimulus than do these regions in the intact hemisphere.

4. No regions other than those known to be excitable in the normal animal were found to be excitable in these preparations.

5. The changes in excitability in the animals operated on in infancy are consistent with the well developed motor performance of such animals during life.

6. They are consistent with functional reorganization within a partially destroyed motor system. There was no evidence of anatomical reorganization.

References

1. Jacobsen, C. F., Taylor, F. V., and Haslerud, G. M. The effect of cardiac sympathetics and adrenalin upon ventricular rhythms induced in cats by inhalation of petroleum ether. *Amer. J. Physiol.*, 1936, *116:* 111–112.
2. Kennard, Margaret A. Cortical reorganization of motor function: Studies on series of monkeys of various ages from infancy to maturity. *Arch. Neurol. Psychiat., Chicago,* 1942, *48:* 227–240.

SWEAT GLAND RESPONSES TO SYMPATHETIC STIMULATION STUDIED BY THE GALVANIC SKIN REFLEX METHOD*

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OUR KNOWLEDGE is still limited regarding the nature, intensity and frequency of the nervous impulses which reach the sweat glands from the sympathetic nervous system. The lack of an adequate method of measuring sweat gland responses may in large part account for this lack of knowledge.

In the following experiments we used the galvanic skin response of cats to measure sweat gland activity produced by electrical stimulation of the sympathetic chain. With this method it proved possible to determine several simple principles regarding the sympathetic nerves and sweat gland relationship.

METHODS

To keep the cats quiet while the records were being taken we placed them in a frame which was originally built for this purpose by Dr. Sarah Tower. A pillory arrangement at one end firmly secured the head and prevented the animal from biting, while the fore and hind legs were either held by an assistant or were tied with strips of muslin bandage to the upright parts of the frame (1).

The electrodes consisted of $\frac{3}{4}$-inch zinc discs covered with a paste made of kaolin and saturated zinc sulphate solution. The paste served to make a good electrical connection between the electrode and the skin. One electrode was fastened by means of a small ear ring clip to the pinna of the ear. The other electrode covered with an even layer of paste was placed over the central pad of one of the hind feet. However, before the electrodes were applied the foot was bandaged with a strip of rubber dam. A small hole in the dam allowed only the large central pad to protrude, thus preventing the paste from coming into contact with the small pads and with the skin between the pads. A second bandage of rubber dam around the foot held the electrode in place over the central pad.

The electrodes were connected to a portable string galvanometer (Cambridge Instrument Company) with which photographic records of the galvanic response from the central pad of the foot were obtained. Since no external current was used, the galvanic response consisted of a difference in potential between the electrodes.

For unknown reasons some cats show little or no galvanic activity even in response to direct stimulation of the sympathetic chain. We found that by taking records of the spontaneous galvanic waves before exposing the sympathetic chain such unresponsive cats could be eliminated. Cats which show active galvanic waves before going under the ether anesthesia will invariably show active galvanic responses to stimulation of the sympathetic chain.

When the galvanic activity of the cats had been satisfactorily established, they were put under a light ether anesthesia without removing the head from the pillory or the electrodes from the feet. The sympathetic chain was exposed retroperitoneally through an incision along the line of the ileum and over the thigh of the hind leg. When the chain between the 2nd and 3rd lumbar ganglia had been freed from the surrounding tissue, it was placed in an insulated bipolor shielded electrode (Harvard Apparatus Company) and the incision was closed in such a way as to avoid any pull on the chain. When the cats are

* This work was carried on under a grant from the John and Mary R. Markle Foundation.

kept warm with a hot bottle, active galvanic responses can be obtained in this way for many hours.

The chain was stimulated with a current from an inductorium (Harvard Apparatus Company) and a 1.5 volt dry cell.

Results

Response to single shocks. Single induced shocks applied to the sympathetic chain near L2 and L3 invariably caused the central pad of the hind foot to give off only single monophasic galvanic responses. In 10 cats the response had an average latent period of 0.6 and a duration of 5.0 sec. The amplitude varied with the intensity of the current as will be shown. Figure 1 shows that the rise of the response was sharp while the fall was relatively gradual.

Response to make and break shocks of varying intensity. Figure 1 gives a typical record. The vertical lines spaced 1 mm. apart show time in $\frac{1}{5}$ seconds,

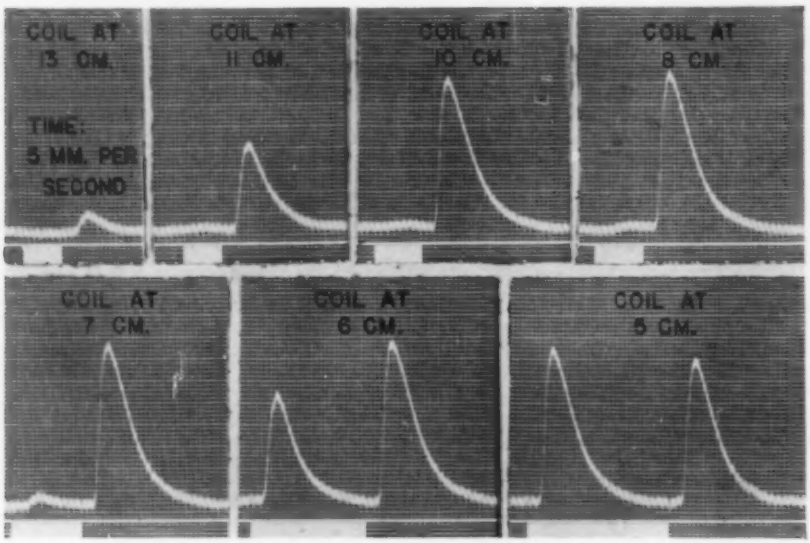

FIG. 1

that is, 5 spaces are equal to 1 sec. The white blocks at the bottom of the record show the make and break of the stimulating circuit. For the first record the secondary coil was set at 13 cm., that is, the weakest current that can be obtained on the Harvard inductorium unless the secondary coil is turned on its axis. The 'make' had no effect; the 'break' produced a small current. With the coil at 11 cm. the 'make' still failed to give any response, while the amplitude of the 'break' response was considerably increased. With the coil at 10 cm. the 'make' again failed to give response, while the 'break' gave a maximum response. At 7 cm. the 'make' gave a small response, and at 6 cm. a fairly large response. At 5 cm. the 'make' response was larger than the 'break.' The latent period did not change with the intensity of the cur-

rent, but it was the same for 'make' or 'break' shocks. The duration of the response varied somewhat with the value of stimulating current, increasing very slightly with an increase in current. The other 9 cats gave essentially the same type of record.

Tetanization of the galvanic response. In these experiments the coil of the inductorium was set at a position which gave an active galvanic response. The inductorium was set for 'make' and 'break' currents. Single combined

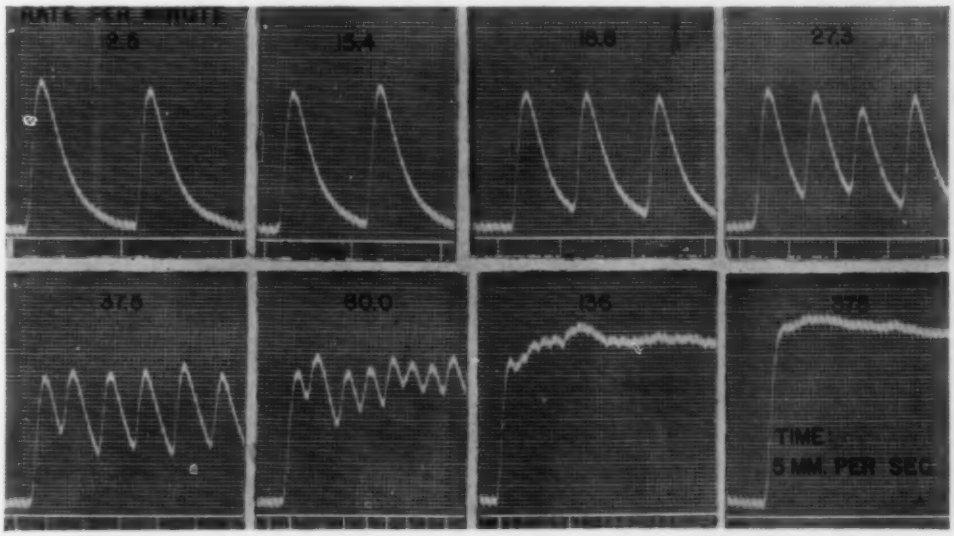

FIG. 2

'make' and 'break' shocks were applied to the sympathetic chain. These shocks were produced by the experimenter by a quick touch to the stimulating key.

Figure 2 gives a typical record. When the shocks were applied at a rate of 12.5 per min. the single complete responses were obtained, so also at a rate of 15.4 per min. At 18.8 per min. the response did not quite return to the base line before a second response started. Progressively as the rate of the shocks increased, the responses showed an increased tendency to tetanization. At 375 per min. the responses were completely tetanized. That the the response would tetanize at this rate could readily have been predicted from the shape and duration of the single response.

The rate of tetanization, 2–6 per sec., is far below that needed to tetanize striped muscle.

DISCUSSION

In these experiments it was found that a single shock applied to the sympathetic chain produced only a monophasic galvanic response. It is of interest that in previous experiments it was found that electric shocks ap-

plied to the cut surface of the spinal cord and brain stem also produced only monophasic currents (2, 3, 4). Biphasic or multiphasic currents were obtained only from stimulation of the intact cortex.

In human beings monophasic currents are obtained from a painful stimulation such as a pin prick, shot, etc. These results would indicate that to the intact human being these stimuli cause only a single discharge to pass down the sympathetic chain to the sweat glands.

With a more prolonged stimulus, such as is produced by emotional excitement, the galvanic response in human beings tends to have more the shape of the curves which show complete tetanization. From this we may conclude that the rate of nervous discharge to the chain under these conditions must be greater than 2–4 per sec. It could not be as low as 6 per sec.; but might be much higher.

Special mention should be made of the great sensitivity of the galvanic response. In some cats a definite response was obtained when the secondary coil of the inductorium was placed not only at the farthest end but also was turned on its axis until it was almost in the vertical position. Under these circumstances the current must have been extremely minute.

Evidence at hand indicates that the galvanic current produced by the stimulation of the sympathetic chain corresponds to a change in permeability of the sweat glands as they become activated, and not necessarily to the amount of moisture present in the sweat gland ducts and on the surface of the skin. If this response depended on the moisture they would have a much longer duration.

Summary

1. Single induced shock applied to the sympathetic chain (L2 and L3) of cats caused a galvanic current to be given off by the large central pad of the hind foot. This response which was monophasic had an average latent period of 0.6 and a duration of 5 seconds.

2. The threshold for 'make' currents was much higher than that for 'break' currents. With larger currents the amplitude of the 'make' response finally surpassed that for the 'break.'

3. Induced shocks at a rate of 136 to 375 per minute or 2 to 6 per second sufficed to obtain complete tetanization of the sweat glands, as indicated by the galvanic responses.

REFERENCES

1. Richter, C. P. Nervous control of the electrical resistance of the skin. *Johns Hopk. Hosp. Bull.*, 1929, *45:* 56–74.
2. Wang, Ging-Hsi, and Richter, C. P. Action currents from the pad of the cat's foot produced by stimulation of the tuber cinereum. *Chin. J. Physiol.*, 1928, *2:* 279–284.
3. Langworthy, O. R., and Richter, C. P. The influence of efferent cerebral pathways upon the sympathetic nervous system. *Brain*, 1930, *53:* 178–193.
4. Richter, C. P. Galvanic skin reflex from animals with complete transection of the spinal cord. *Amer. J. Physiol.*, 1930, *93:* 468–472.

LOCALIZATION OF THE SALIVATORY CENTER IN THE MEDULLA OF THE CAT*

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INTRODUCTION

THE EXISTENCE of a salivatory center in the lower brain stem has never been disputed. However, investigators have not been able to agree on its precise anatomical location and its axonic course. In 1902, Kohnstamm (6), using the method of retrograde degeneration, described in dogs a submaxillary nucleus, with its large cells of the anterior horn type widely distributed in the reticular formation at the caudal level of the facial nucleus. Later, Kohnstamm (7) found a parotid nucleus between the inferior olive and the nucleus ambiguus. The efferent fibers were largely crossed. In 1909, Yagita and Hayama (17) repeated these observations and found that after sectioning the chorda tympani a group of cells of the visceral motor type underwent chromatolysis. These cells were located in the lateral reticular formation, at the level of the facial nucleus, ventral to the lateral vestibular nucleus of Deiters and medial to the spinal trigeminal nucleus. Yagita (16) described a parotid nucleus forming the caudal prolongation of his submaxillary nucleus. Both groups of degenerate cells were homolateral to the cut nerves. Salivary secretion has also been repeatedly noted by a number of investigators on stimulation of the medulla (see review by Langley, 9), but the available data give little help in further identification of the center. It was thought, therefore, that a careful examination of the lower brain stem with the aid of the Horsley-Clarke stereotaxic instrument might yield valuable information as to the location of the center.

While this investigation was underway, Chatfield (2) and recently Magoun and Beaton (10) reported similar studies in cats and monkeys respectively. The results of our study are in general agreement with their reports, but certain differences will be noted.

METHODS

Thirty-five cats were included in this series. The animals were anesthetized with 35–40 mg. of nembutal per kg. of body weight, intraperitoneally. After the cerebellum was exposed, the Horsley-Clarke instrument was fastened to the cat's head. The submaxillary and parotid ducts on the right side were cannulated with fine glass cannulas, and each duct was connected to a horizontally-placed, graduated capillary tube. The secretion was recorded in cm. and the rate of flow was later converted into cc. per min. The right cervical sympathetic nerve was cut in order to isolate the parasympathetic effects.

In the early experiments, no parotid secretion was obtained either on stimulation of the medulla or by injection of pilocarpine. Since it was believed that the ear bar of the

* A preliminary report of this work was presented before the American Physiological Society, Boston. *Fed. Proc. Amer. Soc. exp. Biol.*, 1942, 1, 88–89.

Horsley-Clarke instrument might have pushed the peri-parotid tissue downward, thus occluding the duct, a modification of the point of fixation was made (Fig. 1). This consisted of a round bar holder, which could be adjusted in various directions, held securely on the frame of the instrument, anterior to the usual ear bar holder. The tip of the round bar tapered to a small drill head which was fixed to the posterior part of the zygomatic process. After this had been done, the right ear bar was withdrawn. Thus the new attachment transferred the point of fixation from the right external acoustic meatus to the right zygoma without changing the coordinates. After this modification a parotid flow was easily obtained.

The stimulating electrode was made of two thin nichrome wires, completely insulated except at the tips, which were about 0.2 mm. apart. It was introduced into the brain stem

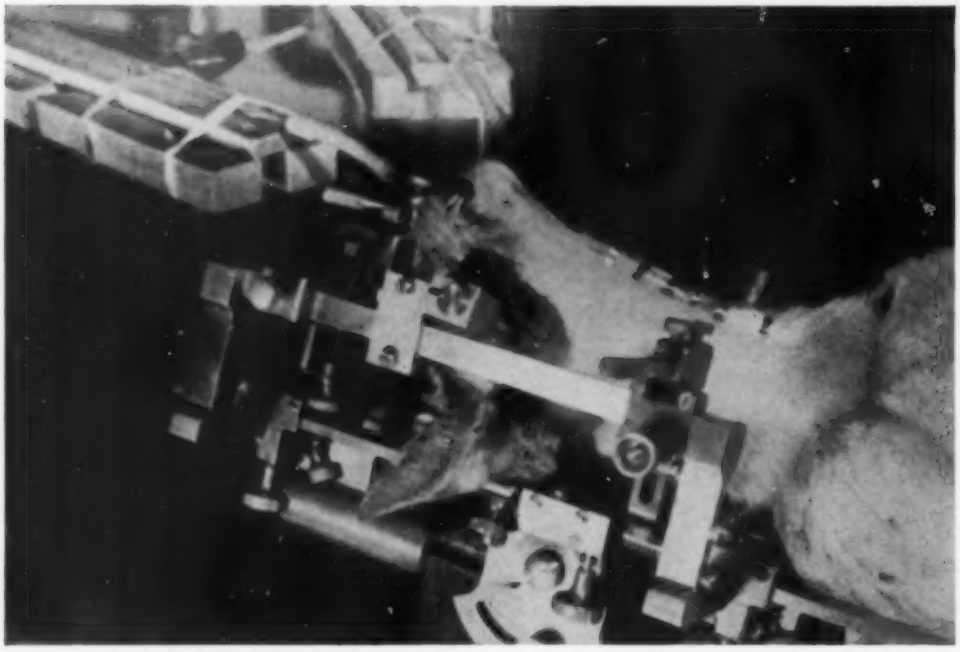

FIG. 1. Photograph to show the arrangement of the experiment with the modified attachment of the Horsley-Clarke stereotaxic instrument to the right zygomatic process of a cat. The right ear bar has been withdrawn.

at a forward inclination of 11 degrees. The stimulating current was provided by a Harvard inductorium having a dry cell (1.5 V.) and a small rheostat (0.5Ω) in the primary circuit, with the secondary coil set at 13.5 cm. For each 15-second stimulus the coordinates and the amount of secretion from both glands were recorded. At the end of the experiment the brain was perfused with 10 per cent formalin and serial sections stained by the Weil method. The region that yielded the submaxillary or parotid secretion was determined by microscopic examination. This is indicated by circles or triangles respectively in six composite diagrams (Fig. 2).

It must be noted that although the submaxillary and parotid ducts were cannulated only on the right side, both sides of the brain stem were explored. Hence, any crossing of fibers in the brain stem is revealed. In the accompanying diagrams submaxillary points in the right half of the medulla are represented on the right side. Parotid points are transposed to the side opposite their actual location in the medulla. In this way the overlapping of symbols is minimal.

Results

The most rostral level included in the exploration was at the anterior border of the pons. Stimulation of the brain stem at the pontile level even with strong currents produced no appreciable flow of saliva from either the submaxillary gland or the parotid gland, whereas stimulation of the responsive regions at the successive levels in the medulla even with weak

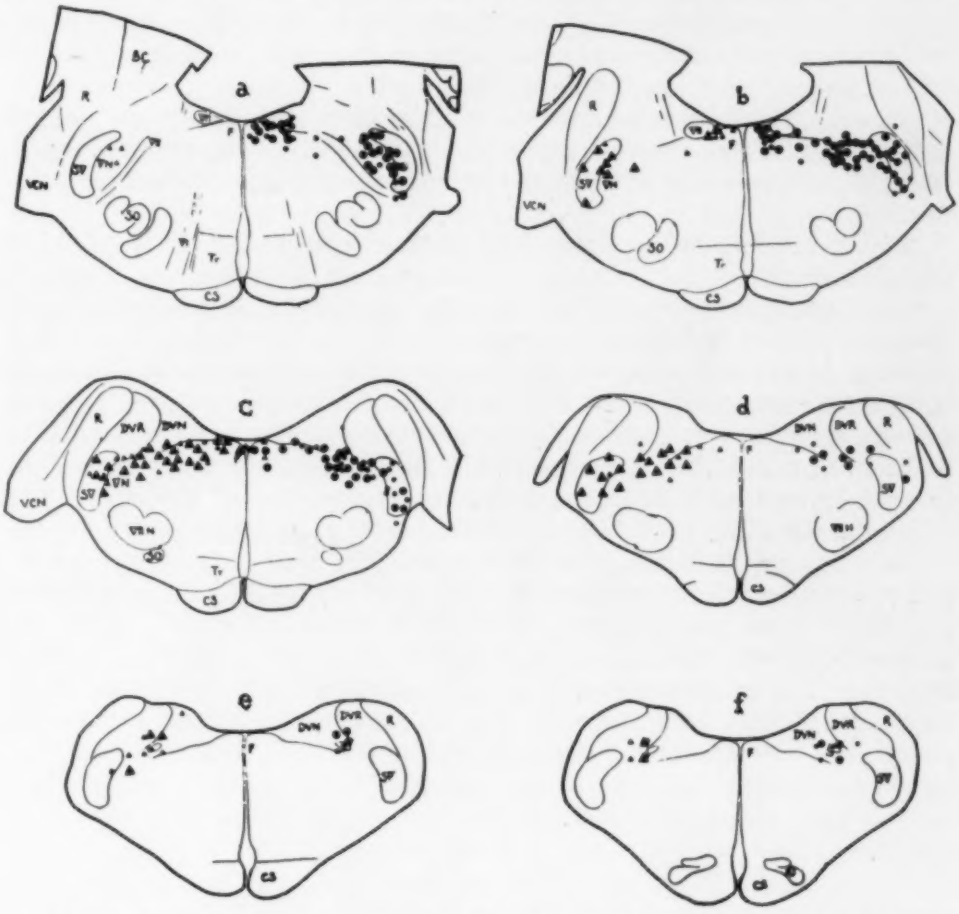

Fig. 2. Diagrammatic representation of results obtained upon stimulation of the medulla of 35 cats. The sections are about one mm. apart. Each large solid circle or triangle represents a response of salivary flow at a rate of more than 0.25 cc. per min., and each small solid circle or triangle, at a rate of 0.1 cc. to 0.25 cc. per min. Secretion of less than 0.1 cc. per min. is not represented. Circles indicate responses from the right submaxillary gland, and triangles, responses from the right parotid gland but transposed to the opposite side. Abbreviations are: BC—brachium conjunctivum; CS—cortico-spinal tract; DVN—dorsal or medial vestibular nucleus; DVR—descending vestibular root; F—medial longitudinal fasciculus; IO—inferior olive; R—restiform body; S—Solitary fasciculus; SO—superior olive; SV—Spinal tract of the trigeminal nerve; Tr—trapezoid body; VCN—ventral cochlear nucleus; VM—spinal nucleus of the trigeminal nerve; VI—abducens nerve; VII—facial nerve; VIIN—facial nucleus.

currents induced a copious flow of saliva. The swallowing or respiratory movements which occasionally accompanied salivary secretion were not causally related to the salivary flow. This was shown by experiments in which stimulation of an appropriate region yielded salivation associated with deglutition or respiratory movements, while stimulation of the corresponding contralateral region yielded similar extraneous responses without comparable salivary secretion.

After a 15-second period of stimulation at a favorable location, as much as 1.2 cc. per min. of secretion was collected from the submaxillary gland, and as much as 1.1 cc. per min. from the parotid. The locations which, upon stimulation, yielded a secretion of more than 0.25 cc. per min. are represented in the diagrams by large solid circles (submaxillary) and large solid triangles (parotid), representing an average of 0.5 cc. per min. and 0.4 cc. per min. respectively.

A typical submaxillary flow would commence about 2 seconds following stimulation; that from the parotid usually had a longer latency. The flow for the first 15-second period was usually most rapid, decreasing for each 15-second period thereafter. Consequently the flow per min. as calculated from the first 15-second period is greater than the amount of saliva collected if the stimulus actually lasted for one minute. The after-discharge was sustained for about 3–4 seconds, and rarely longer. For the less favorable regions (0.1 cc. to 0.25 cc. per min.), the latency was slightly longer and in many instances the flow slowed down considerably before the end of the 15-second stimulus.

A striking distribution of the responsive locations of the submaxillary points in two groups was revealed at the bulbar level. This is shown in Fig. 2a, in which a medial group lies in the vicinity of the facial genu, and a lateral group in the area corresponding to the spinal trigeminal nucleus and tract. The intervening region was surprisingly silent when stimulated. At a level about one mm. caudad (Fig. 2b), the two responsive regions adjoined. The area medial and slightly dorsal to the spinal trigeminal nucleus and its caudal extension form a very excitable region, which, on stimulation, produced an abundant submaxillary secretion. This area corresponds to the dorso-lateral portion of the lateral reticular formation and is believed by Papez (12, p. 222) to constitute the rostral component of the salivary nucleus. In the section through the facial nucleus and superior olive and at the level of nervus intermedius (Fig. 2c) the medial region was less responsive than the corresponding rostral area. At this level (Fig. 2c) the responsive points were most numerous in the dorsolateral portion of the lateral reticular formation, although scattered points were present in the spinal trigeminal area. At more caudal levels (Fig. 2e and 2f) salivary secretion was occasionally obtained when the area of the solitary fasciculus and its nucleus was stimulated.

The configuration of the parotid points is very similar to that of the submaxillary, except that the corresponding parotid regions appear roughly one or two mm. more caudally. Profuse secretion was not obtained following

weak stimulation at the level of the outgoing limb of the facial nerve (Fig. 2a). More caudally, at the level of the facial nucleus and the superior olive (Fig. 2c) parotid secretion was more easily obtained. It was noted that stimulation of the same locus often produced secretion from both submaxillary and parotid glands. In one experiment a definite but small parotid secretion was obtained when the contralateral medial floor of the fourth ventricle was stimulated (Fig. 2c). At the next level through the middle portion of the facial nucleus and through the glossopharyngeal nerve (Fig. 2d) the responsive region corresponds to the dorsolateral portion of the lateral reticular formation and to the spinal trigeminal nucleus and tract. At the lower levels (Fig. 2e and 2f) stimulation of the area of the solitary fasciculus occasionally was found to yield good secretion. It is interesting to note that stimulation of the area between the inferior olive and the nucleus ambiguus designated by Kohnstamm as the inferior salivatory nucleus (8) did not cause any definite flow of saliva from the parotid gland.

Responses from both the submaxillary and the parotid glands were obtained when portions of the homolateral medulla were stimulated. Scanty secretion from either gland, however, was obtained when the contralateral side was stimulated. Even when strong currents were used, no flow greater than 0.25 cc. per min. was observed. Under such conditions the area of homolateral reactivity was considerably scattered, and vigorous respiratory changes and contractions of various muscles occurred.

The mechanism of the salivary secretion obtained following central stimulation has also been investigated. For instance, the intravenous injection of 0.1 mg. of atropine promptly and completely stopped the salivary secretion produced by medullary stimulation. In a number of experiments the chorda tympani was sectioned before it joins the lingual nerve. No submaxillary secretion could be elicited by stimulation of the medulla at a point which previously yielded a copious flow of saliva.

Discussion

It has long been known that the salivary glands receive their innervation from two general sources: the sympathetic and the parasympathetic. Stimulation of either nerve supply produces secretion. In this series of experiments the cervical sympathetic fibers have been severed. Consequently the responses obtained cannot be due to stimulation of the medullary sympathetic or descending hypothalamic pathways. It is also well established that the vasodilation following the stimulation of the chorda tympani has no causal connection with the secretion of saliva by the gland in consequence of the stimulation (1). The fact that in our experiments a small dose of atropine abolishes the secretion produced by stimulation of responsive points in the medulla also indicates that vaso-dilation, which remains active after atropine,* is not the primary factor causing secretion. Hence, it is

* Henderson and Loewi (4) found that atropine in small doses paralyzed only the secretory but not the vaso-dilator fibers of the chorda tympani.

reasonable to attribute the salivatory effect obtained on stimulation of the medulla in these experiments to the excitation of the parasympathetic secretory components, including the center itself.

According to Miller (11) the threshold for eliciting salivary secretion by stimulation of the floor of the fourth ventricle or by reflex stimulation of chorda-lingual or glossopharyngeal nerves is low, whereas the threshold for salivary secretion induced by stimulation of the gastric vagus or sciatic nerves is high. These observations suggest that stimulation of the intramedullary courses of the afferent "taste" fibers should yield salivary secretion.* In our experiments stimulation of the region corresponding to the solitary fasciculus and its nucleus was found occasionally to yield abundant secretion from both the submaxillary and the parotid glands. The salivary secretion resulting from stimulation of the spinal trigeminal area appears to be more complicated in that, while stimulation of the rostral portion of the trigeminal area produced abundant secretion, stimulation of the more caudal portion induced no salivary secretion. The presence of a number of rootlets of the nervus intermedius and the glossopharyngeal nerve which pass only through the rostral spinal trigeminal area suggests that these fibers might account for the responses obtained on stimulation. However, on careful examination, it appears doubtful that a copious salivary secretion would follow a punctate stimulation of a few of the widely distributed efferent rootlets. Furthermore, the isolated groups of positive points from which salivary secretion was obtained in the most rostral regions of the spinal trigeminal area (Fig. 2a) would not have been found if the salivary secretion had resulted from the excitation of efferent fibers passing through the stimulated area.

Various investigators have suggested that different branches of the trigeminal nerve terminate at different levels of the spinal trigeminal tract. For example, it is believed that the mandibular branch occupies only the rostral portion of the spinal trigeminal tract and does not extend down to the caudal region (3). This conception would explain the refractoriness of the caudal portion of the spinal trigeminal area (Fig. 2e and 2f) when it is stimulated with weak currents. The evidence indicates, therefore, that stimulation of the spinal trigeminal tract and nucleus at a specific level (presumably mandibular) produces salivary secretion. Nevertheless, the response obtained on stimulating this area may be the result of excitation of efferent rootlets as well as of the secondary afferent fibers.

The areas dorso-medial (referred to below as medial) and dorsal (referred to below as lateral) to the facial nucleus constitute regions in which the salivatory nuclei may be located. In favor of the medial position is the fact

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* Our experiment differs from that of Miller in that we used nembutal anesthesia instead of decerebration. It is known that barbiturates decrease the salivary secretion (14), but this action is thought to be exerted on the secretory cells. Pearcy and Weaver (13) found that barbital in quantities sufficient for surgical anesthesia causes little or no depression of other visceral reflexes.

that other general visceral efferent neurones, such as the Edinger-Westphal nucleus of the third cranial nerve and the dorsal motor nucleus of the tenth cranial nerve, are located along the axis. However, if this position is accepted, it becomes difficult to explain the abundant submaxillary secretion which results from stimulation of wide areas of the lateral reticular formation directly dorsal to the facial nucleus (Fig. 2c). There is no reason to assume that the efferent and afferent fibers, if scattered over a wide region, would yield, upon stimulation such vigorous responses in a rather circumscribed area. If, on the other hand, the nuclear masses are located in the lateral position, the axons will have to travel, like those from the facial nucleus and the nucleus ambiguus, rostrally and dorso-medially before making their exit. The increased responsiveness in the lateral area may then be readily accounted for by the stimulation of the cells and the efferent fibers after they have made the turn, as well as the secondary afferent fibers from the nucleus of the solitary fasciculus and the spinal trigeminal nucleus. It is recognized that the evidence presented here in favor of the lateral location of the salivatory center is at best presumptive. Nevertheless, this contention is strengthened by Yagita's anatomic studies of the salivatory nuclei in the dog (17) and by Kimmel's recent ontogenic studies of the same nuclei in the rabbit (5).

Summary

The lower brain stem of 35 cats has been stimulated with the aid of the Horsley-Clarke stereotaxic instrument. It was found that a copious salivary flow is easily elicited from the homolateral glands when the medulla is stimulated with a weak current.

Analysis of the responsive locations reveals that salivary secretion can be obtained by stimulation of the intramedullary visceral (oral) afferent system, such as the solitary fasciculus and its nucleus and certain portions of the spinal trigeminal nucleus and tract (mandibular division?). On the efferent side, the distribution suggests that the salivatory nuclear masses might be either in the medial position caudal to the facial genu, or more likely, in the dorso-lateral region of the lateral reticular formation, dorso-medial to the spinal trigeminal nucleus, and dorsal to and at the level of the facial nucleus. In the latter case, the efferent fibers must travel dorso-medially before they turn and make their exit in the ventro-lateral portion of the medulla. In any event, there is no sharp division of the centers of the salivatory nerve fibers carried in the seventh and ninth cranial nerves. The rostral portion supplies the submaxillary glands, and the caudal portion the parotid. There exists an intermediate portion, stimulation of which yields both submaxillary and parotid secretion.

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REFERENCES

1. Carlson, A. J., Greer, J. R., and Becht, F. C. The relation between the blood supply to the submaxillary gland and the character of the chorda and the sympathetic saliva in the dog and the cat. *Amer. J. Physiol.*, 1907, *20:* 180–205.
2. Chatfield, P. O. Salivation in response to localized stimulation of the medulla. *Amer. J. Physiol.*, 1941, *133:* 637–641.
3. Gerard, M. W. Afferent impulses of the trigeminal nerve: the intramedullary course of the painful, thermal and tactile impulses. *Arch. Neurol. Psychiat., Chicago*, 1923, *9:* 306–338.
4. Henderson, V. E., and Loewi, O. Über den Einfluss von Pilocarpin und Atropin auf die Durchblutung der Unterkieferspeicheldrüse. *Arch. exp. Path. Pharmak.*, 1905, *53:* 62–75.
5. Kimmel, D. L. Differentiation of the bulbar motor nuclei and the coincident development of associated root fibers in the rabbit. *J. comp. Neurol.*, 1940, *72:* 83–148.
6. Kohnstamm, O. Der Nucleus salivatorius chorda tympani (nervi intermedii). *Anat. Anz.*, 1902, *21:* 362–363.
7. Kohnstamm, O. Der Nucleus salivatorius inferior und das cranioviscerale System. *Neurol. Zbl.*, 1903, *22:* 699.
8. Kohnstamm, O., and Wolfstein, J. Versuch einer physiologischen Anatomie der Vagusursprünge und des Kopfsympathicus. *J. Psychol. Neurol., Lpz.*, 1907, *8:* 177–203.
9. Langley, J. N. The salivary glands, vol. 1, pp. 475–530 in: *Schäfer's Textbook of physiology*. Edinburgh, 1898.
10. Magoun, H. W., and Beaton, L. E. The salivatory motor nuclei in the monkey. *Amer. J. Physiol.*, 1942, *136:* 720–725.
11. Miller, F. R. On the reactions of the salivary centres. *Quart. J. exp. Physiol.*, 1913, *6:* 57–72.
12. Papez, J. W. *Comparative neurology*. New York, Thomas Y. Crowell, 1929, 507 pp.
13. Pearcy, J. F., and Weaver, M. M. A study of the bulbo-spinal reflexes in dogs and cats under barbital anesthesia. *Amer. J. Physiol.*, 1927, *82:* 47–50.
14. Stavraky, G. W. Effect of amytal on the autonomic nervous system as indicated by the salivary glands. *J. Pharmacol.*, 1931, *43:* 499–508.
15. Wang, S. C. Localization of the salivatory "points" in the lower brain stem of cat. *Fed. Proc. Amer. Soc. exp. Biol.*, 1942, *1:* 88–89.
16. Yagita, K. Weitere Untersuchungen über das Speichelzentrum. *Anat. Anz.*, 1909, *35:* 70–75.
17. Yagita, K., and Hayama, S. Über das Speichelsekretionscentrum. *Neurol. Zbl.*, 1909, *28:* 738–753.

LOCALIZATION OF ENZYMES IN NERVES. II. RESPIRATORY ENZYMES

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INTRODUCTION

THE HIGH concentration of choline esterase (ChE) at the neuronal surface, which may be demonstrated in the giant axon of the squid, and the parallelism found between the concentration of the enzyme and the E.M.F. of the action potential in electric organs suggest that the release of acetylcholine (ACh) is intrinsically connected with the electrical changes which occur during nerve activity (3, 8, 10). Investigations were therefore initiated to study the distribution of other enzymes known to be involved in the metabolism of nerve. Experiments showed that approximately 90 per cent of succinic dehydrogenase and of the succinic oxidizing system is located in the axoplasm, in contrast to the localization of ChE. There was no evidence that a high concentration of these enzymes occurred at or near the neuronal surface (9). The concentration of vitamin B_1 (as diphosphothiamin), on the other hand, is many times higher on the neuronal surface than in the axoplasm.

In the present paper further observations on the distribution of respiratory enzymes in the giant axon of the squid are described.

METHODS

Cytochrome oxidase. The O_2 uptake was measured by the usual manometric method of Barcroft-Warburg. For the determination of cytochrome oxidase, small conic vessels were used, the total volume being only about 5.0 cc. and the K_{O_2} under conditions of the experiments 0.30–0.35. Tissue was ground in 0.1 M phosphate buffer of pH 7.4 and 1.0 cc. of the suspension was put into the vessels. The homogenizer of Potter and Elvejhem was used to grind it. During the grinding the tube containing the tissue was kept in ice water. Axoplasm was put directly into the buffer without grinding since it dissolves rapidly in isotonic solution. In 0.2 cc. H_2O p-phenylenediamine dihydrochloride was added to give a final concentration of 0.03 M. Cytochrome c, prepared according to the method of Keilin and Hartree (4) was added in 0.2 cc. of buffer, the final concentration being 1 mg. per cc. Controls were run without tissue.

Pyruvic oxidase. The respiration with pyruvate or glucose as substrate was measured in conic vessels with a center well for the KOH. The total volume was about 7 cc., the K_{O_2} with 1.0–2.0 cc. of fluid present was 0.5–0.6 cc.; 0.1 cc. of 8 per cent KOH were put into the center well. The tissue was either minced or ground in 0.1 M phosphate buffer of pH 7.4. The final concentration of sodium pyruvate was 0.05 M. In a few experiments with minced tissue no other substances were added. In all the other experiments the following

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additions were made: cytochrome c, diphosphopyridine nucleotide,* cocarboxylase (diphosphothiamin, prepared by Merck) and adenosinetriphosphate, prepared from rabbit muscle according to the method of Lohman (6).

Pyruvic dehydrogenase. The method of Quastel and Wheatley (11) was used, in which the reduction of ferricyanide by hydrogen atom leads to the formation of acid. In bicarbonate solution this liberates CO_2 which is estimated manometrically. The method was used as described for succinic dehydrogenase (9). The failure of Quastel and Wheatley to determine pyruvic dehydrogenase may be due to insufficient concentration of some coenzymes necessary for this enzyme system (5, 11). The substances used were those mentioned for the work with pyruvic oxidase.

Preparation of the tissue. In view of the high sensitivity of some respiratory enzymes special precaution has to be used for the preparation of the material. The trunk was rapidly cut and put into sea water of about +2°C. The preparation of the isolated giant axon requires considerable time and some preliminary experiments indicated that during this time the rate of respiration decreased. The isolation of the giant axon was therefore abandoned and the following procedure was adopted: The whole trunk after having been cooled at 2°C. for 5–8 minutes was put on a cool glass plate and the axoplasm extruded. The respiration of the axoplasm could then be compared with that of the whole trunk as well as with that of the remaining tissue consisting of small nerve fibers, connective tissue and sheath.

Estimation of dry weights. The dry weights used for the calculation of the Q_{O_2} values were as follows: For the head ganglion 200 mg. per gm. fresh weight, determined by Nachmansohn and B. Meyerhof (8); for the axoplasm 100 mg. per gm. fresh weight, determined by Bear and Schmitt (2); for the whole trunk 140 mg. per gm. and for the remaining tissue 148 mg. per gm. fresh weight. The figure for the whole trunk is based on the assumption that it has about the same dry weight as a lobster nerve trunk, for which Schmitt, Bear and Silver obtained a value of 140 mg. per g. (13). For the remaining tissue the figure found by Bear and Schmitt for the envelope of the giant fiber was taken. Although it is possible that the actual values vary slightly from these figures, the difference could alter the final result only slightly.

Results

A. *Cytochrome oxidase*

The distribution of the succinic dehydrogenase found in the giant fiber suggests that the bulk of the respiratory enzyme system is located in the axoplasm. This is however only indirect evidence. The succinic-fumaric system may not be necessary in all oxidative processes. On the other hand, there is general agreement that the first activation of oxygen of physiological

Table 1. Cytochrome oxidase in nerve tissue of the squid

Tissue ground in 1.0 cc. of 0.1 *M* phosphate buffer, pH = 7.4. 0.2 cc. of p-phenylenediamine, final concentration 0.03 *M*. 0.2 cc. of cytochrome c, 1.0 mg. per cc. Time of observation 30 min. t = 23°C.

Exp. No.	Head Ganglion		Trunk Containing Giant Axon		Axoplasm		Remaining Tissue	
	Mg. Tissue	Q_{O_2}	Mg. Tissue	Q_{O_2}	Mg. Tissue	Q_{O_2}	Mg. Tissue	Q_{O_2}
1	63.0	*− 9.5*	77.0	*−0.98*	59.0	*−2.86*	61.0	*−0.25*
2	63.0	*− 9.3*	143.0	*−0.90*	43.0	*−3.42*	54.0	*−0.74*
3	39.0	*−13.0*			30.0	*−3.40*		

* Kindly provided by Dr. M. E. Krahl, Lilly's Research Laboratories.

significance in living cells occurs through the cytochrome-cytochrome oxidase system. It appeared therefore necessary to determine the distribution of cytochrome oxidase.

Cytochrome oxidase has been determined (i) in the head ganglion of the squid as sample of nerve tissue in which cell bodies and synapses are located, (ii) in the whole trunk containing the giant axon, (iii) in the axoplasm extruded from the trunk and (iv) in the tissue of the trunk which remains after the extrusion of the axoplasm.

The results are summarized in Table 1. The head ganglion has a remarkably high rate of oxygen uptake for the tissue of a cold blooded animal. The

Table 2. Pyruvic oxidase in nerve tissue of the squid

Minced tissue. In the experiments Nos. 1–3 with the head ganglion and Nos. 1 and 2 with the trunk the tissue was put into 0.8 cc. of 0.1 molar phosphate buffer of pH 7.4. 0.1 cc. of sodium pyruvate was added, its final concentration being 0.05 *M*. In the other experiments the tissue was put into 0.5 cc. phosphate buffer and besides sodium pyruvate the following additions were made: 0.1 cc. of cytochrome c, final concentration = 1 mg. per cc.; 0.12 cc. of adenosinetriphosphate, f.c. = 3 mg. per cc.; 0.1 cc. of diphosphopyridinenucleotide, f.c. = 10 μg. per cc.; 0.1 cc. of diphosphothiamin, f.c. = 50 μg. per cc. In centric well 0.1 cc. of 8 per cent KOH. Time of observation 30 min. t = 23–24°C.

Exp. No.	Head Ganglion		Trunk Containing Giant Axon		Axoplasm		Remaining Tissue	
	Mg. Tissue	Q_{O_2}	Mg. Tissue	Q_{O_2}	Mg. Tissue	Q_{O_2}	Mg. Tissue	Q_{O_2}
1	42.0	*−6.7*	47.0	*−1.00*	57.0	*−0.34*	73.0	*−0.37*
2	32.0	*−8.9*	44.0	*−1.22*	73.0	*−0.92*	123.0	*−0.88*
3	51.0	*−8.4*	77.0	*−1.27*				
4	89.0	*−9.7*	184.0	*−1.40*				

figures obtained with the whole trunk and with the axoplasm and remaining tissue separately offer evidence that the greatest part of the cytochrome oxidase is localized in the axoplasm. The Q_{O_2} values of the axoplasm (the O_2 uptake per mg. of tissue dry weight) are about three times as high as those of the whole trunk. The difference between the whole trunk and the axoplasm is even more marked than in the case of succinic dehydrogenase. In the remaining tissue the values are lower than in the whole trunk. It has to be borne in mind that while most of the axoplasm is removed from the giant axon, some still remains attached to the sheath. Moreover the small fibers of the trunk retain their axoplasm. Part of the activity of the remaining tissue has therefore to be attributed to the axoplasm remaining in the trunk.

B. *Pyruvic oxidase*

Since pyruvate, or glucose, increases acetylcholine formation in brain in aerobic conditions, the enzymes necessary for the oxidation of these two substances are of special interest in connection with the problem investigated.

The results obtained with pyruvate as substrate are given in Table 2. The rate of O_2 uptake in minced head ganglion is nearly the same as if p-phenylenediamine is used as substrate, the Q_{O_2} values being -7.0 to -9.0. This indicates that the concentrations of all the enzymes and coenzymes necessary for the intermediate reactions between the first step of pyruvic acid oxidation and the cytochrome-cytochrome oxidase system are sufficiently high so that they are not limiting factors. The oxidation of pyruvic acid in dialyzed extracts of brain requires, according to Banga, Ochoa

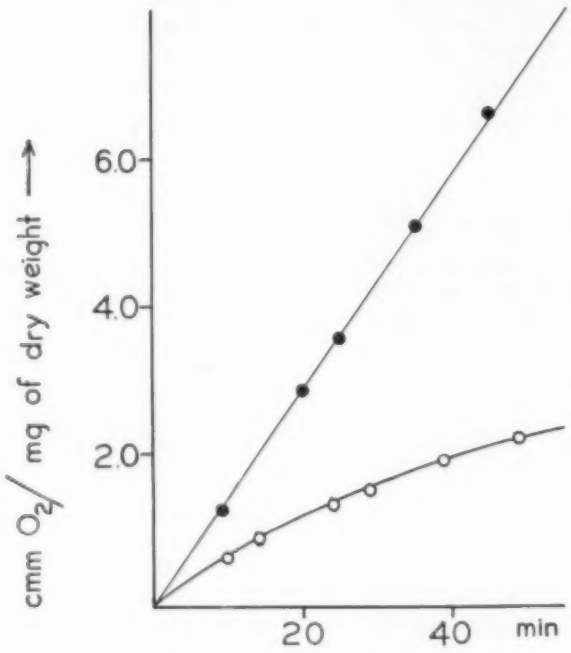

FIG. 1. Pyruvate oxidation in the head ganglion of squid. Abscissae: cmm. O_2 per mg. of tissue dry weight. Ordinates: time in min. ●—● minced, ○—○ homogenized tissue. 0.5 cc. of 0.1 M phosphate buffer, pH = 7.4. 0.1 cc. of sodium pyruvate, final concentration (f.c.) 0.05 *M* 0.1 cc. of cytochrome c.f.c. 1 mg. per cc. 0.2 cc. of adenosinetriphosphate, f.c. 3 mg. per cc. 0.1 cc. of diphosphothiamin, f.c. 50 μg per cc. 0.1 cc. of diphosphopyridine nucleotide, f.c. 10 μg per cc.

and Peters (1), at least the following substances: adenylic acid or adenosinepolyphosphate, diphosphothiamin (cocarboxylase), inorganic phosphate in a concentration of 0.05–0.1 molar, a C_4-dicarboxylic acid such as succinic, fumaric or malic acid in catalytic amounts, magnesium ions and probably, although the evidence is not conclusive, diphosphopyridine nucleotide. But since the oxidation of pyruvic acid in minced ganglion is already as high as the activity of cytochrome oxidase permits, addition of these compounds cannot have any marked effect, and the Q_{O_2} of Experiment 4 is in fact the same as in the experiments without these additions.

When however the ganglion is ground, the rate of pyruvic acid oxidation falls off rapidly, even in the presence of all the factors known to be of consequence in this process. Figure 1 shows the difference between ground and minced tissue. In this experiment the first reading was made about one hour after the animal was killed. In an experiment with ground ganglion in which the preparation was made with high rate of speed, the first reading was

made 20 min. after death, the oxidation rate during the first 30 min. was nearly as high as in minced tissue. In the second half hour the rate of the oxidation went down rapidly and did not differ from that of the experiment in Fig. 1. Even so the compounds added seem to have some effect as in the brain dispersions of Banga, Ochoa and Peters. In the experiment presented

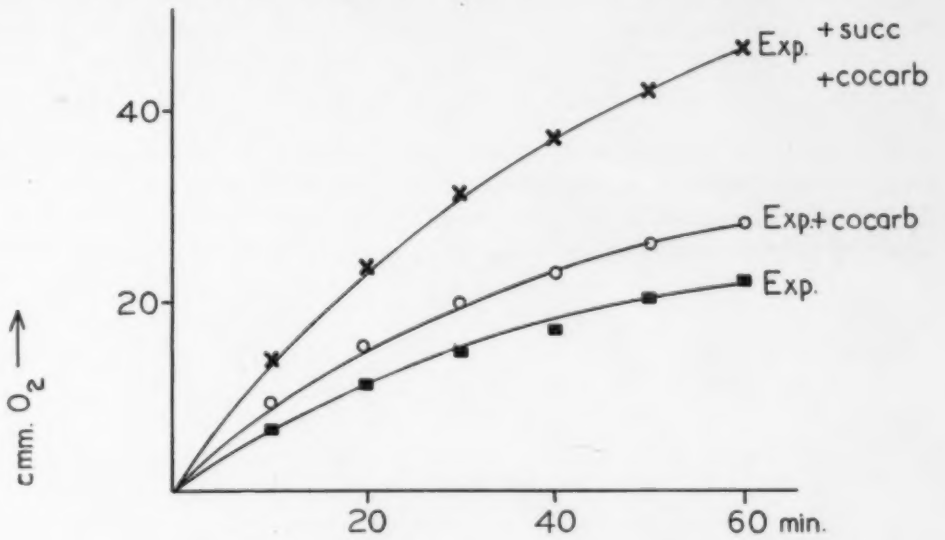

FIG. 2. Effect of diphosphothiamin and succinate on pyruvate oxidation with an homogenized suspension of the head ganglion of the squid. The concentration of the substances added was the same as in the experiments shown in Fig. 2 and 3.

in Fig. 2, the rate of O_2 uptake is lowest in the vessel containing pyruvate, adenosinetriphosphate, diphosphopyridine nucleotide and cytochrome c. The rate rises if diphosphothiamin is added and even more if, besides that, a C_4-dicarboxylic acid (succinic acid) is present.

The difference between pyruvic oxidation in ground and minced tissue indicates that some enzymes necessary for this process are rather fragile and rapidly lose their activity if the cell is destroyed, or there is still an unknown factor which is not present in sufficiently high concentration in homogenized tissue suspension. It can therefore not be expected that pyruvic acid oxidation in the axoplasm is measurable, because the axoplasm dissolves rapidly in isotonic solution. The Q_{O_2} values are indeed low.

If glucose is used as substrate with minced ganglion, the rate is the same as with pyruvate (Table 3).

C. *Pyruvic dehydrogenase*

In brain preparations pyruvic acid can be broken down to some extent by dehydrogenation to carbon dioxide and acetic acid (11). Diphosphothiamin strongly stimulates pyruvic acid dehydrogenation in a homogenized suspen-

Table 3. Respiration of the head ganglion of squid

Substrate: glucose, 0.01 molar in 0.1 molar phosphate buffer, pH = 7.4. Minced tissue. Time of observation 30 min. t = 23°C.

Exp. No.	1	2	3	4	5
Mg. Tissue Fresh Weight	42.0	36.0	42.0	36.0	34.0
Q_{O_2}	−8.2	−8.0	−6.3	−7.0	−8.2

sion of the head ganglion. The effect is shown in Fig. 3. In the presence of adenosinetriphosphate, succinic acid and diphosphopyridine nucleotide, the rate of CO_2 production in the presence of pyruvate is only slightly increased over the rate of control. But if cocarboxylase is added the increase of the

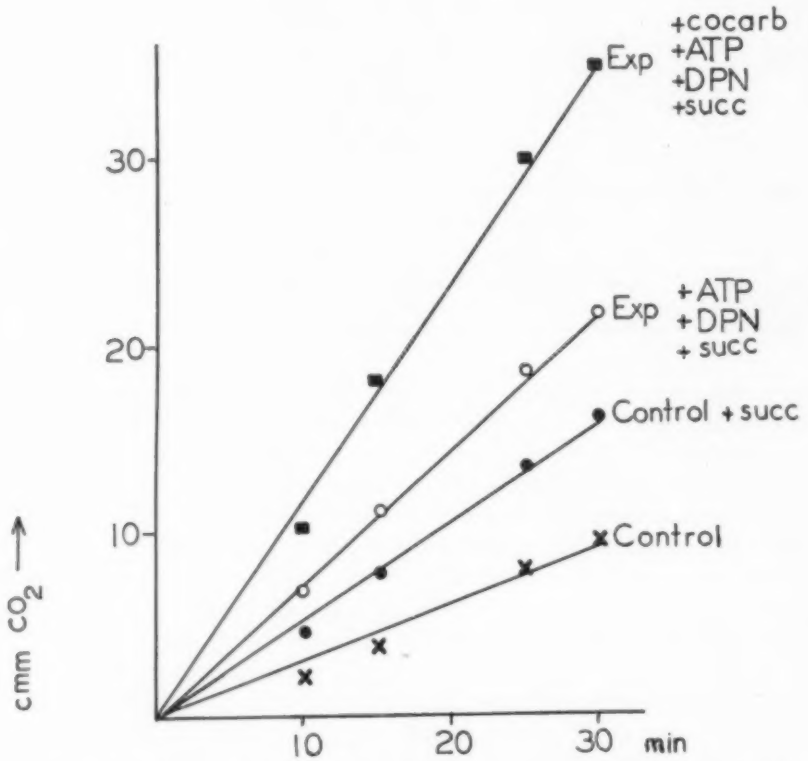

FIG. 3. Effect of diphosphothiamin on pyruvate dehydrogenation with an homogenized suspension of the head ganglion of the squid. In each vessel 1.0 cc. of suspension in bicarbonated Ringer. Concentration of substances added: 0.2 of sodium pyruvate, f.c. 0.05 *M*. 0.1 cc. of sodium succinate, f.c. 0.001 *M*. 0.2 cc. of adenosinetriphosphate (ATP), f.c. 3 mg. per cc. 0.2 cc. of diphosphopyridine nucleotide (DPN), f.c. 10 μg per cc. 0.1 cc. of diphosphothiamin (cocarboxylase), f.c. 50 μg per cc. 0.1 cc. of potassium ferricyanide in the side bulb.

rate of CO_2 production is about 3 times as high as without this compound. The concentration of diphosphothiamin was 50 μg per cc., a concentration not far from the possible or even probable physiological concentration at or near the neuronal surface.

The presence of adenosinetriphosphate has only a slight effect, as shown in the experiment presented in Fig. 4.

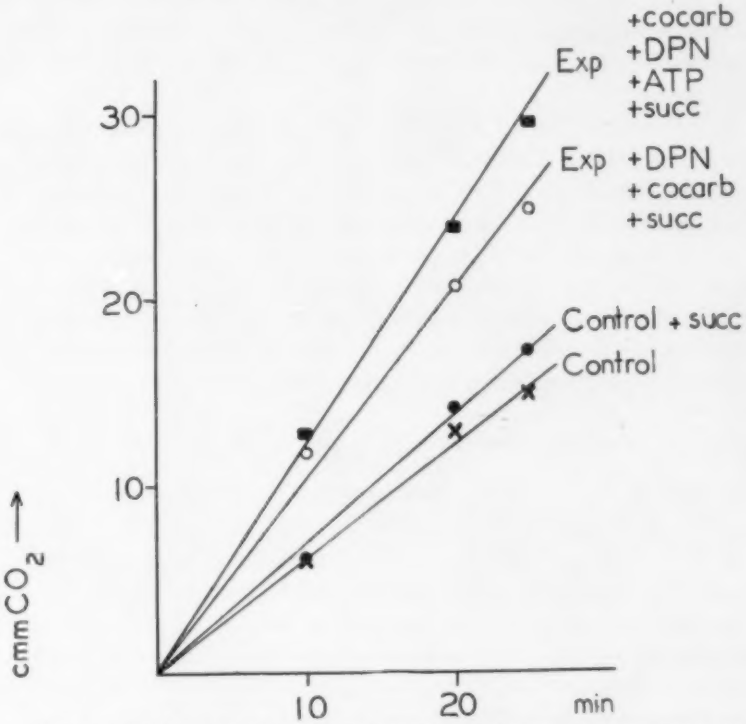

FIG. 4. Effect of adenosinetriphosphate (ATP) on pyruvate dehydrogenation with an homogenized suspension of the head ganglion of the squid. Concentrations used are the same as in the experiment shown in Fig. 3.

DISCUSSION

The observations here described on the distribution of cytochrome oxidase demonstrate its high concentration in the axoplasm of the nerve fiber as compared with the low concentration in the nerve sheath. Since the physiological activation of oxygen occurs through the cytochrome-cytochrome oxidase system, this distribution appears significant. It does not indicate any direct or immediate connection between oxidation and the action potential, although oxidation must be the final energy source as for all cell processes. The localization of choline esterase at the neuronal surface in contrast to that of respiratory enzymes becomes even more significant. It is worthy of note that the rate of O_2 uptake is, in absolute values, low even in

the axoplasm as compared with other cells whereas the rate of acetylcholine metabolism is extremely high in the sheath.

The oxidation of pyruvic acid, necessary for acetylcholine formation in brain tissue, occurs in the head ganglion and the trunk containing the giant axon at approximately the same rate as the O_2 uptake with p-phenylenediamine. This indicates that the intermediate steps of pyruvic acid oxidation occur at a rate limited only by that of the O_2-uptake through the cytochrome-cytochrome oxidase system. Direct evidence, however, could not be brought since the oxidation of pyruvic acid falls off rapidly if the cell structure is not intact.

The increase of the rate of pyruvic acid dehydrogenation in presence of diphosphothiamin is of special interest. According to Long about 20 per cent of the pyruvic acid removed by oxidation in brain, can be accounted for as acetic acid, whereas the greater part is completely oxidized to carbon dioxide and H_2O. In view of the high concentration of diphosphothiamin at the neuronal surface it is possible that a higher percentage of pyruvate is there transformed into acetic acid by dehydrogenation. Whether this is of physiological significance for the formation of acetylcholine can not be decided at present and the problem requires further investigation.

Summary

1. The distribution of cytochrome oxidase in nerve tissue of the squid has been studied. In the head ganglion the concentration is remarkably high, the Q_{O_2} at 23°C. being -9.0 to -13.0. In the axoplasm extruded from the trunk containing the giant axon the concentration is lower, but relatively high compared with that of the remaining tissue. This finding is evidence for the previous assumption that the bulk of the respiratory enzymes is confined to the axoplasm while in contrast practically all of the choline esterase is found at the neuronal surface.

2. Oxidation of pyruvic acid in the minced head ganglion occurs at a rate similar to that of p-phenylenediamine, the Q_{O_2} being about -7.0 to -9.0. On the other hand, in a ground suspension of the head ganglion the activity of pyruvic oxidase falls off rapidly even if the following substances known to be of consequence in pyruvic acid oxidation are added: cytochrome c, adenosinetriphosphate, diphosphothiamin, diphosphopyridine nucleotide and succinate. In the axoplasm the activity of pyruvic oxidase was small, although in the whole trunk if minced, the rate of O_2 uptake is about the same with pyruvate as with p-phenylenediamine. The axoplasm dissolves rapidly in isotonic solution. Therefore the low Q_{O_2} values must be attributed to the rapid loss of actvity if the cell structure is destroyed.

3. Pyruvic dehydrogenation is strongly increased in the presence of diphosphothiamin. In view of the concentration of this coenzyme at the neuronal surface this incomplete breakdown, yielding acetic acid and carbon dioxide, may be of significance for the formation of acetylcholine.

REFERENCES

1. BANGA, I., OCHOA, S., and PETERS R. A. Pyruvate oxidation in brain. VII. Some dialysable components of the pyruvate oxidation system. *Biochem. J.*, 1939, *33:* 1980–1996.
2. BEAR, R. S., and SCHMITT, F. O. Electrolytes in the axoplasm of the giant nerve fibers of the squid. *J. cell. comp. Physiol.*, 1939, *14:* 205–215.
3. BOELL, E. J., and NACHMANSOHN, D. Localization of choline esterase in nerve fibers. *Science*, 1940, *92:* 513–514.
4. KEILIN, D., and HARTREE, E. F. Preparation of pure cytochrome *c* from heart muscle and some of its properties. *Proc. roy. Soc.*, 1937, *B 122:* 298–308.
5. LIPMANN, F. Metabolic generation and utilization of phosphate bond energy. *Adv. Enzymology*, 1941, *1:* 99–162.
6. LOHMANN, F. Darstellung der Adenylpyrophosphorsäure aus Muskulatur. *Biochem. Z.*, 1931, *233:* 460–469.
7. MANN, P. J. G., and QUASTEL, J. H. Vitamin B_1 and acetylcholine formation in isolated brain. *Nature*, 1940, *145:* 856–857.
8. NACHMANSOHN, D., and MEYERHOF, B. Relation between electrical changes during nerve activity and concentration of choline esterase. *J. Neurophysiol.*, 1941, *4:* 348–361.
9. NACHMANSOHN, D., and STEINBACH, H. B. Localization of enzymes in nerves. I. Succinic dehydrogenase and vitamin B_1. *J. Neurophysiol.*, 1942, *5:* 109–120.
10. NACHMANSOHN, D., COX, R. T., COATES, C. W., and MACHADO, A. L. Action potential and enzyme activity in the electric organ of *Electrophorus electricus* (Linnaeus). I. Choline esterase and respiration. *J. Neurophysiol.*, 1942, *5:* 499–515.
11. OCHOA, S. Cocarboxylase, pp. 17–42 in: *Symposium on the biological action of the vitamins.* Chicago, 1942.
12. QUASTEL, J. H., and WHEATLEY, A. H. M. On ferricyanide as a reagent for the manometric investigation of dehydrogenase systems. *Biochem. J.*, 1938, *32:* 936–943.
13. SCHMITT, F. O., BEAR, R. S., and SILBER, R. H. Organic and inorganic electrolytes in lobster nerves. *J. cell. comp. Physiol.*, 1939, *14:* 351–356.

MECHANISM OF TEMPORAL FUSION EFFECT OF PHOTIC STIMULATION ON ELECTRICAL ACTIVITY OF VISUAL STRUCTURES

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INTRODUCTION

IN CONTRAST with its high resolving power in space, the resolving power in time of the mammalian visual system is relatively low. Thus the range of frequencies of intermittent light that man can distinguish from steady light is from 4 or 5 cycles per sec. (c.p.s.) at low intensities to about 55 c.p.s. at high intensities (11). Since it is well known that sensory nerves can transmit discrete impulses at rates above this upper limit, it has come to be rather generally assumed, in the absence of experimental evidence to the contrary, that the limiting factor in temporal resolving power must be the retina. Thus "retinal lag" or "persistence" has long been made the basis of many visual phenomena from the fusion (freedom from flicker) of motion pictures when projected at 15 to 20 c.p.s. to chromatic after-images. The essentially retinal locus of the fusion mechanism has, moreover, gained substantial support in recent years from the demonstration by Hecht and his co-workers that Talbot's Law, governing the apparent brightness of intermittent light at the point of fusion, may be derived from the equations of a reversible photochemical reaction, which had previously been evolved to describe certain photosensory behavior of the clam, *Mya arenaria* (10, 11, 12).

It would seem to be a useful working hypothesis that the primary phenomena of fusion are dependent upon the maximum rate at which the *slowest* element in the visual system can be reactivated from the periphery. Such an hypothesis not only permits a relatively direct experimental approach but does not obviate the possible operation of secondary mechanisms such as may be required, for example, to account for such phenomena of flicker *constancy* as described by Bartley (3).

As one step in our analysis of this problem, experiments have been carried out on monkey, whose fusion mechanism is known to have several important features in common with that of man (6, 13).

In previous papers we have shown that in line with the observations of Bartley (1, 4) and Bishop (5) on cat and rabbit, the electrical activity recorded from the region of the occipital lobe of monkey may be modified in certain characteristic ways by intermittent photic stimulation (8, 9). The present report extends this investigation to the effects of such stimulation on the optic nerve, lateral geniculate body, tectum mesencephalon, optic

radiation and cerebral cortex, particularly with reference to the differential rates at which these structures can be reactivated from the periphery.

METHOD

The results are based upon experiments carried out on 9 adult monkeys (*Macaca mulatta*).* The majority of these animals had had operations on the internal ear through the mastoid bone several weeks before our acute experiment.

The technique and apparatus for securing simultaneous electroencephalograms and a record of photic stimulation have been described previously (8, 9). After anesthetizing the animals with nembutal administered intraperitoneally, the scalp was reflected and the calvarium removed from the occipital lobe. The dura was opened and reflected medially. The animal was then placed on a board facing the source of light. In most experiments both pupillary and accommodative reflexes were eliminated by installation of 0.5 per cent solution of scopolamine hydrochloride into the conjunctival sacs of both eyes. This, however, proved to be unnecessary, and satisfactory results were obtained with undilated pupils. Blinking movements were eliminated by continuous use of lid retractors; this procedure was also found to be superfluous. An Adrian-Bronk concentric dipolar needle electrode was passed manually through the middle portion of the first temporal convolution into the lateral geniculate body on one or both sides. When the activity of the tectum mesencephali was studied, a needle electrode was inserted along the tentorium cerebelli. A similar needle electrode was inserted into the optic nerve by passing it through the inner canthus of the eye along the medial margin of the orbit until it reached the optic nerve.

Most of the recording was done by means of a three-channel ink-writing electroencephalograph. For greater accuracy in determining time relations, additional recording was made on bromide paper with a moving loop oscillograph.

The position of the electrodes was controlled anatomically by removing the brain and sectioning it in slabs 1 mm. in thickness after formalin fixation.

RESULTS

The results will be discussed under the headings of the individual anatomical structures.

1. *Cerebral cortex*

From leads placed upon the striate cortex on either the medial or lateral surface of the cerebral hemisphere electrical responses were obtained from photic stimulation of the retina. Leads from any other cortical area have not given such responses; even when placed on the parastriate area there were no responses. The response may be of several types. At slower speeds of stimulation (up to 8–9 per sec.) usually the response consists of two waves, one following the "on" signal and the other the "off". At slow speeds the "on" response is usually greater than the "off" response. But as the speed increases the two may become of equal amplitude (Fig. 1E). With still further acceleration the "off" effect drops out and the resultant effect is a series of approximately sinusoidal waves corresponding to the rate of stimulation (Fig. 1).

Effect of frequency. The cortical rhythm may be driven up to a frequency of 34 per sec. Above this level it proved impossible to drive the cortical potentials with the luminous intensities used. Possibly with higher intensities the cortex would respond to individual flashes at higher frequencies. The highest intensity at the eye of the monkey has been 30 foot candles,

* It is a pleasure to acknowledge the kindness of Dr. John R. Lindsay in placing these animals at our disposal for terminal experimentation.

which, using an episcatistor having a light dark ratio of 1:2 means that the effective flicker intensity was 10 foot candles.

At a low light intensity it was possible to produce driving with low frequencies whereas high frequencies were ineffective. Thus in one preparation using an intensity of 4-foot candles driving could be obtained at a frequency of 8 but not of 17 per sec. Increasing the intensity at the latter frequency produced good driving.

Intensity. The importance of the intensity of the photic stimulus is indicated by the fact that in general the higher the frequency, the more intense the stimulus required. We have obtained driving at an intensity below $\frac{1}{4}$ foot candle. With low intensities driving resulted with low frequencies. The higher ranges of intensity have not been explored.

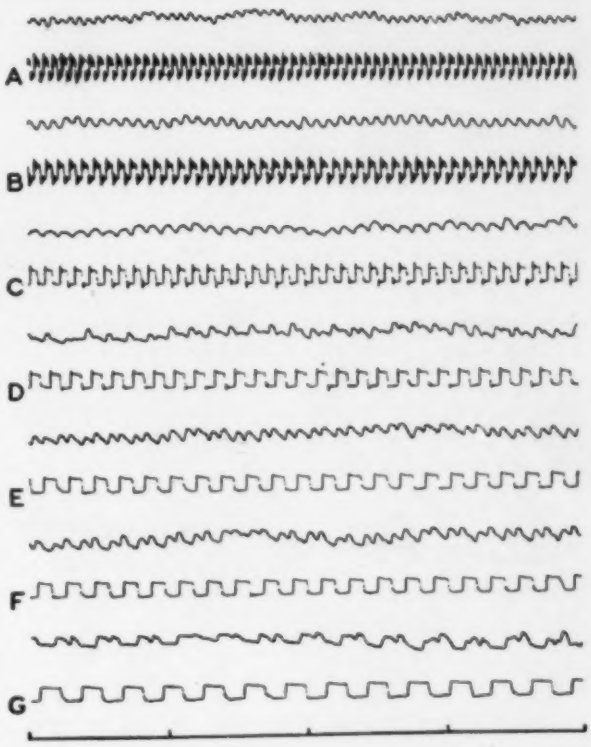

FIG. 1. A series of electrocorticograms of the striate area showing the driving effect of photic stimulation of the retina at frequencies from 3.5 cycles per sec. (G) to 15 per sec. (A). The distinct "off" and "on" effect disappears between 7.5 c.p.s. (D) and 9.5 c.p.s. (C).

2. *Lateral geniculate body*

Electrical responses in the lateral geniculate body follow regularly upon photic stimulation of the retina at frequencies up to 59.1 per sec. Beyond this point driving could not be elicited, although the activity of the lateral geniculate body is distinctly changed from that of the resting state. The responses are usually spike-like but at times assume wave form. At times a frequency in the geniculate develops at twice the rate of retinal stimulation, but more often and especially with higher frequencies the geniculate rate is half that of the photic stimulation (Fig. 2).

The lateral geniculate response occurs to minimal intensities of light but is more pronounced to brighter photic stimuli.

3. *Optic nerve*

Responses obtained from the optic nerve are in many respects similar to those from the lateral geniculate body. They will follow stimuli up to a

frequency of 62 per sec. Higher photic intensities cause augmentation of the response obtained from the optic nerve (Fig. 3).

Tectum mesencephali. With needle electrodes in the tectum mesencephali (superior colliculus) good driving responses to photic stimulation were obtained. The amplitude of these responses is not so great as that from the lateral geniculate body. That it is not an artefact due to pick-up of spread is evident from the fact that no responses occur when the electrode is placed in the adjacent corpus callosum.

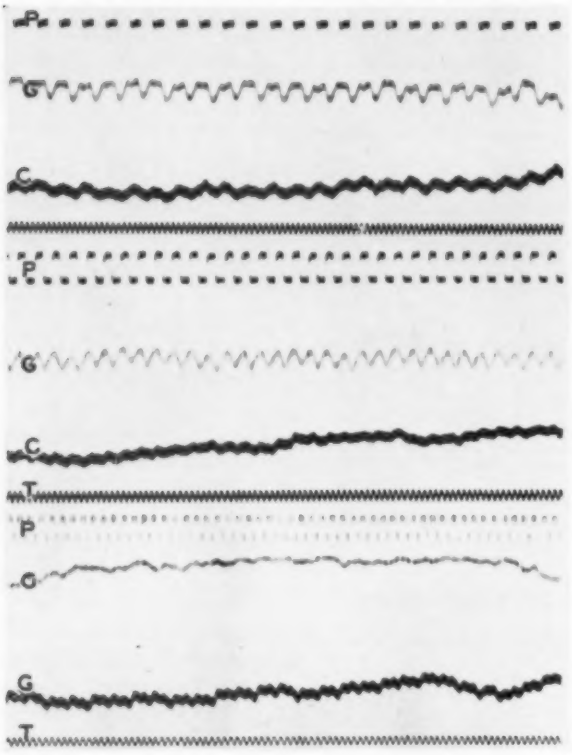

FIG. 2. A series of records from the striate cortex (C) and the lateral geniculate body (G) to show the driving effect at different frequencies. The top record is at 23 c.p.s. the middle at 32.5 c.p.s. and the lowest one at 59 c.p.s. In the first two records both the cortex and lateral geniculate body respond to the photic stimuli (P), but in the third record the cortex has assumed its own rhythm and only the lateral geniculate body responds. At the bottom of each record is a time signal (T) indicating 1/100 sec. The tracings were made on bromide paper with the loop oscillograph.

Optic radiation. On a number of occasions we have attempted to place a needle in the optic radiations but the responses have never been satisfactory. Whether this is because the fibers of the radiation are more diffuse than at the geniculate level or is the result of the type of impulse passing over these fibers is not evident at this time.

DISCUSSION

Localization of driving effect. Evidence of cortical localization of the driving effect was revealed by the fact that driving at rates up to 34 c.p.s. could be obtained *only* when the leads were located on the striate cortex. No driving could be elicited from the parastriate area (cf. 15, 16). Further

investigation suggests that photic stimuli may modify the activity of areas other than the striate cortex. This point is now being studied in more detail.

Effect of intensity. The effect of intensity, is in line with our previous findings based upon recording from leads attached to the scalp of the unanesthetized monkey (8, 9). However, the range of frequencies through which intensity is effective is greater in the present instance where recording was from leads placed directly in contact with particular brain structures and with the animal maintained under relatively deep anaesthesia than in the previous experiments.

Differential driving rates for a given intensity. The most striking feature of the present results lies in the marked difference at which, for a given intensity of light, the various visual structures could be reactivated (driven) from the periphery. With a luminous flux (at the eye of the animal) of 10 fc. the optic nerve and lateral geniculate body drove regularly throughout a range of from 1 to 62 c.p.s. and 1 to 59 c.p.s. respectively (2). The striate cortex on the other hand, followed regularly at 1 c.p.s. but could never be driven above 34 c.p.s. Since this latter value falls within the range at which Brecher (6) found intermittent light to *fuse* for monkey, it is possible that a fusion mechanism provided by the cortex constitutes a physiological barrier to driving at higher frequencies. Whether this is definitely the case must await further investigation since it is possible that the nembutal anaesthesia, which we employed, acted to suppress the driving rate of cortical structures while sparing, relatively at least, more peripheral structures. Also, a satisfactory driving effect from the optic radiations has not yet been obtained. It is significant, however, that driving of the optic nerve and lateral geniculate body occurred at a rate as high as 62 and 59 c.p.s. respectively. This is well above the maximum values for fusion, found by Hecht and Verrijp in man (11). Furthermore, Brecher's results indicate that for the range of intensities employed by us, fusion occurs for the monkey at several cycles per

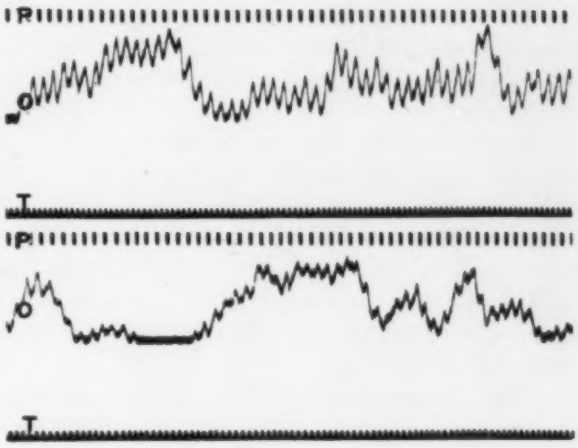

FIG. 3. Two records taken from the optic nerve (O) showing the response to individual photic stimuli (P) at a frequency of 54.2 (above) and 62 (below) per sec. A time signal (T) indicating 1/100 sec. is at the bottom of each record. Both tracings were made on bromide paper with a loop oscillograph.

second *below* that for man. If the velocity of a reversible photochemical reaction in the retina (as suggested by Hecht, 11) establishes a maximum limit beyond which fusion occurs, it seems that *supramaximal* values would be required for driving the optic nerve and lateral geniculate body at a rate of 62 and 59 c.p.s. respectively.

Possible pupillary effect eliminated. Most of the determinations of maximum fusion frequency in man have employed either an artificial or the natural pupil. However, Halstead (7) could demonstrate no significant difference in critical fusion frequency in a normal subject with maximal pupillary dilatation produced by scopolamine. As mentioned previously in our statement of method, mydriasis had no influence on the driving effect in monkey.

Other evidence of neural basis of fusion. Recently reported observations by Klüver (14) indicate that, in contrast with the behavior of a normal monkey, the differential response of a monkey in which both occipital lobes have been removed is not disturbed by substituting an intermittent light of moderate intensity for a continuous one. This was true when the intermittent rate was as slow as 4 c.p.s. (light to dark ratio = $\frac{1}{3}$). This might be due to marked shift or lowering of critical fusion frequency associated with removal of the occipital lobes. As support for such an interpretation, however, it should be proved to be impossible to establish a differential response in the monkey lacking both occipital lobes to two stimuli equated as to luminous flux but differing in rate of intermittency, for example, 10 c.p.s. versus 5 c.p.s.

The observations of Seitz (17) that strychnine applied to one eye of human subjects raises the critical fusion frequency for that eye without affecting the other eye would seem to be in line with a neural interpretation of the fusion mechanism, unless it can be shown that strychnine markedly augments photochemical activity. In Seitz' experiments the elevation of critical fusion frequency under strychnine was of the same order as the depressing effect on fusion frequency of acute exposure to the anoxia associated with a simulated altitude of 20,000 feet. It should perhaps be noted that his conclusion that the effect of strychnine "cancels" the effect of anoxia is somewhat misleading. That it may *mask* the effect of anoxia on critical fusion frequency is probably as much as can be concluded from his results.

Summary

The driving effect of intermittent photic stimulation on the electrical activity of the optic nerve, lateral geniculate body, tectum mesencephalon, optic radiations and cortex has been explored in monkey (*Macaca mulatta*). It was found:

(i) At an intensity of 10 fc., the optic nerve and lateral geniculate body could be driven at a maximum rate of 62 c.p.s., and 59 c.p.s. respectively, a

rate that is well above the maximal critical fusion frequency for man and probably for monkey;

(ii) The cortex of the striate area could be driven at a maximum rate of 34 c.p.s.

(iii) While a driving effect could be obtained from the tectum mesencephali and from the optic radiations, it was not sufficiently stable to permit a determination of maximum rate.

(iv) The possiblility is considered that our findings indicate a fusion mechanism in the cortex, which limits the temporal resolving power of the primate visual system.

REFERENCES

1. BARTLEY, S. H. Temporal and spatial summation of extrinsic impulses with the intrinsic activity of the cortex. *J. cell comp. Physiol.*, 1936, *8:* 41–62.
2. BARTLEY, S. H. The neural determination of critical flicker frequency. *J. exp. Psychol.*, 1937, *21:* 678–686.
3. BARTLEY, S. H. Subjective flicker rate with relation to critical flicker frequency. *J. exp. Psychol.*, 1938, *22:* 388–394.
4. BARTLEY, S. H., and BISHOP, G. H. The cortical response to stimulation of the optic nerve in the rabbit. *Amer. J. Physiol.*, 1933, *103:* 159–172.
5. BISHOP, G. H. Electrical responses accompanying activity of the optic pathway. *Arch. Ophthal.*, 1935, *14:* 992–1019.
6. BRECHER, A. Die Verschmelzungsgrenze von Lichtreizen beim Affen. *Z. vergl. Physiol.*, 1935, *22:* 539–547.
7. HALSTEAD, W. C. A note on the Bartley effect in the estimation of equivalent brightness. *J. exp. Psychol.*, 1941, *28:* 524–528.
8. HALSTEAD, W. C., KNOX, G. W., and WALKER, A. E. Modification of cortical activity by means of intermittent photic stimulation in the monkey. *J. Neurophysiol.*, 1942, *5:* 349–356.
9. HALSTEAD, W. C., WOOLF, J. I., KNOX, G. W., and WALKER, A. E. Effects of intensity and wave length on driving cortical activity in monkeys. *J. Neurophysiol.*, 1942, *5:* 483–486.
10. HECHT, S. Vision: II. The nature of the photoreceptor process, pp. 704–828 in: MURCHISON, C., *Handbook of general experimental psychology*. Worcester, Mass., 1934.
11. HECHT, S., and VERRIJP, C. D. Intermittent stimulation by light: III. The relation between intensity and critical fusion frequency for different retinal locations. *J. gen. Physiol.*, 1933, *17:* 251–268.
12. HECHT, S., and WOLF, E. Intermittent stimulation by light. I. The validity of Talbot's Law for *Mya*. *J. gen. Physiol.*, 1932, *15:* 369–389.
13. KLÜVER, H. *Behavior mechanisms in monkeys*. Chicago, Univ. Chicago Press, 1933, xvii, 387 pp.
14. KLÜVER, H. Functional significance of the geniculo-striate system. *Biol. Symposia, Visual Mechanisms*, 1942, *7:* 253–299.
15. KORNMÜLLER, A. E. Die bioelektrischen Erscheinungen architecktonischer Felder der Grosshirnrinde. *Biol. Rev.*, 1935, *10:* 383–426.
16. MARSHALL, W. H., TALBOT, S. A., and ADES, H. W. Cortical response of the anesthetized cat to gross photic and electrical afferent stimulation. *J. Neurophysiol.*, 1943, *6:* 1–15.
17. SEITZ, C. P. The effects of anoxia on visual function. *Arch. Psychol.*, 1940, *36*, 1–38.

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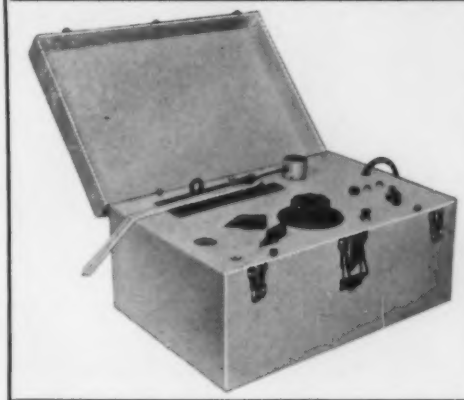

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